FACULTY OF SCIENCES

Poly(2-oxazoline)s

as matrix excipient for oral drug formulations

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PREFACE AND ACKNOWLEDGEMENTS

In my opinion, a PhD is a four-year journey in which a lot of things happen, either good or bad. On the one hand you learn how to cope with challenging Research and development projects, team work, gather experience in a very specific topic and learn how to deal with presenting your own work. On the other hand, a research path does not always follow your mind and you never know how experiments will end-up and eventually meet your expectations. However, you want to try and predict your outcome of the experiments based on your experiences and knowledge. In the end, good results are satisfying, but the strange or bad results will give you the opportunity to think outside of the box, drive you to look for explanations and will get you the achievements you look for in a PhD. Nevertheless, it is of utmost importance that you have a group of people supporting, during this amazing journey, in order to reflect on your own goals, courage and faith in all the things you do!

First of all, I want to thank my promotor, Richard Hoogenboom, whom supported me along the great five years I have been working in the Supramolecular Chemistry group. Since I´ve started my master thesis, I felt like he gave me the chance to grow as a researcher and a person, which has been very important to me. After my thesis, he gave me the confidence and trust to pursue a PhD, which I´m very grateful for until today. Besides the work as a PhD student I had a lot of opportunities to go abroad for conferences and get the chances to evolve and choose my own path between the lines of the PhD work. I see him not only as a supervisor, but also as a true friend who is not afraid of sharing a drink together and talk about other things in life, besides work. I honestly want to say a big THANK YOU, for all the achievements I could realize during my PhD time, because of Richard.

Secondly, the support of the supramolecular chemistry group, as a close team around me, was essential in becoming the person who I am today. I especially want to thank some persons who made it possible to enjoy my life as a PhD student every day. Thank you Jim, for sharing the office, lab, bike rides, honey moon suites … and discussions during the legendary Tea times! It has been an honor to work with you and I´m sure our paths will cross again along the way of our careers! Also, I want to thank Bart for our amazing time during conferences *e.g.* in Boston and Brazil, hardcore lab discussions and memorable evenings in Ghent, which needs no detailed explanation 😉. Of course I also want to thank Joachim, whom I met during my first

year in University, and since then became the start of a long lasting friendship following me along the road. I´m also really happy he joined the Supra team, so we could share and prolong our memorable moments also in our work environment. More related to work experience, I want to gratefully thank Bryn Monnery, who learned me all the tips and tricks involved with the polymerization of 2-oxazolines, including becoming an expert in schlenk line and vacuum techniques. In addition, I want to thank Gertjan, to learn me all the things about Claudia, aka our size-exclusion chromatography instrument. Of course I want to thank all the other people in the Supra group, including Kathleen, Qilu, Maji, Lenny, Mathias, Annelore, Jente, Ondrej, Michal, Bera, Zhanyao, Xiaowen, Glenn, Victor J, Victor DLR, Martin, Luismi, Ali and of course the many other students that visited the group throughout the years of my PhD.

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A final important step for me in becoming a doctor, existed in finding the right partner who you want to share the rest of your life with. Long story short it meant the end of a long relationship, which I regret but sometimes life changes and so do people. A new love story began, which I´m happily sharing with Annelore. Baby, I really want to thank you, for your endless support and believe during the final year of my PhD, which I truly love you for! I also really enjoyed the way we could keep personal and work life separated, there it is not always obvious. I really hope we can keep on telling our stories to each other and share our life's together.

Last but not least I want to thank my family and friends, who supported me always during my choices along my studying career, especially my parents, Luc en Christel, and my two sisters, Marijke en Dorien, who always gave me the support, warmth and kindness I needed.

ABBREVIATIONS LIST

Abbreviation	Description
2,6-DFOx	2,6-Difluoro-2-phenyl-2-oxazoline
3EPOx	2-(3-Ethylpentyl)-2-oxazoline
A	Frequency factor
AA	Acetic acid
ACN	Acetonitrile
ADC	Antibody drug conjugate
Alkyl-PAOx	Poly(2-alkyl-2-oxazoline)s
ALS	Automatic liquid sampler
API(s)	Active pharmaceutical ingredient(s)
Aq	Aquazol®, *i.e.* ill-defined PEtOx
Aryl-PAOx	Poly(2-aryl-2-oxazoline)s
ATRP	Atom transfer radical polymerization
BCS	Biopharmaceutics classification system
bp	Boiling point
C2MestOx	2-Methoxycarboxyethyl-2-oxazoline
C3MestOx	2-Methoxycarboxypropyl-2-oxazoline
cBuOx	2-c-Butyl-2-oxazoline
cGMP	Current good manufacturing practice
CH_3CN	Acetonitrile
$CH_4NHBocOx$	2-Oxazoline monomer with amino *tert*-butoxycarbonyl group
CMC	Chemistry manufacturing controls

Abbreviations list

cPentOx	2-c-Pentyl-2-oxazoline
cPrOx	2-c-Propyl-2-oxazoline
CROP	Cationic ring-opening polymerization
Đ	Dispersity, *i.e.* molar mass distribution
DAD	Diode array detector
DC	Degree of conversion
DCM	Dichloromethane
DCS	Development classification system
DMA	*N,N*-dimethyl acetamide
DMA-SEC	Size-exclusion chromatography with *N,N*-dimethyl acetamide as liquid phase
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	Dimethyl sulfoxide
dn/dc	Refractive index increment
DNA	Deoxyribonucleic acid
DP	Degree of polymerization
DSC	Differential scanning calorimetry
DTA	Differential temperature analysis
E_a	Activation energy
EHOx	2-(3-Ethylheptyl)-2-oxazoline
EMA	European medicine agency
EPOx	2-(1-Ethylpentyl)-2-oxazoline
EtOAc	Ethyl acetate
FA	Formic acid
FBT	Fenofibrate
FDA	Food and drug administration

Abbreviations list

FID	Flame ionization detector
FLU	Flubendazole
GC	Gas chromatography
GI	Gastrointestinal
GPC	Gel permeation chromatography
HBF_4PhOx	2-Phenyl-2-ozazolinium tetrafluoroborate salt
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol
HME	Hot-melt extrusion
HME/IM	Hot-melt extrusion coupled to injection moulding
HMPT	Hexamethylphosphoramide
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropylmethylcellulose
***i*BuOx**	2-*i*-Butyl-2-oxazoline
IM	Injection moulding
***i*PrOx**	2-*i*-propyl-2-oxazoline
IR	Infrared
IUPAC	International union of pure and applied chemistry
k_i	Initiation rate constant
k_p	Propagation rate constant
k_t	Termination rate constant
LCSC	Lower critical solution concentration
LCST	Lower critical solution temperature
ln (M_0/M_t)	Ln Monomer consumption
LS	Light scattering
M:I	Monomer to initiator ratio, *i.e.* DP

Abbreviations list

MALS	Multi-angle light scattering
MEC	Minimum effective concentration
MeOH	Methanol
MeOTs	Methyl *p*-toluene sulfonate
M_n	Number average molecular weight
M_p	Peak average molecular weight
MP	Metoprolol
MPS	Metoprolol succinate
MPT	Metoprolol tartrate
MS	Mass spectrometry
MSC	Maximum safe concentration
MTF	Metformin
MW	Molecular weight
M_w	Weight average molecular weight
n.a.	Non applicable
n.d.	Not determined
***n*ButOx**	2-*n*-Butyl-2-oxazoline
***n*Hexox**	2-*n*-Hexanyl-2-oxazoline
NMR	Nuclear magnetic resonance
***n*NonOx**	2-*n*-Nonyl-2-oxazoline
Nosylate	*p*-Nitrobenzenesulfonates
***n*PentOx**	2-*n*-Pentyl-2-oxazoline
P(M)MA	Poly((methyl) methacrylate)
PAOx (POx, POz)	Poly(2-alkyl/aryl-2-oxazoline)s
PBS	Phosphate buffer saline
PCI	Polymer chemistry innovations®

Abbreviations list

PEG, PEO	Poly(ethylene glycol), Poly(ethylene oxide)
PEG-VA-CL	Poly(ethylene glycol-co-vinyl acetate-co-vinyl caprolactam) graft copolymer
PEI	Poly(ethylene imine)
PEtOx	Poly(2-ethyl-2-oxazoline)
PEtOx-EI	Poly(2-ethyl-2-oxazoline)-stat-(ethylene imine)
PhCl	Chlorobenzene
$PhNO_2$	Nitrobenzene
PhOMe	Anisole
PMA	Poly(methacrylate)
PMeOx	Poly(2-methyl-2-oxazoline)
P*n*PrOx	Poly(2-*n*-propyl-2-oxazoline)
PPhOx	Poly(2-phenyl-2-oxazoline)
P*sec*ButOx	Poly(2-*sec*-butyl-2-oxazoline)
PVOH	Poly(vinyl alcohol)
PVP	Poly(vinyl pyrrolidone)
Ref.	Reference
RID	Refractive index detector
rpm	Revolutions per minute
rt	Room temperature
S	Solubility
$S(H_2O)$	Solubility in water
SD	Solid dispersion
SEC	Size-exclusion chromatography
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid

Abbreviations list

SIF	Simulated intestinal fluid
SIM	Sacrificial initiator method
SRF	Sustained release formulations
std	Standard
std. dev.	Standard deviation
TCC	Thermo-statted column compartment
T_{cp}	Cloud point temperature
TFF	Tangential flow filtration
T_g	Glass transition temperature
TGA	Thermo-gravimetric analysis
THEO	Theophylline
T_m	Melting temperature
TMS	Trimethylsilane
TMSCl	Chloromethyl(trimethyl)silane
$T_{process}$	Processing temperature
UCST	Upper critical solution temperature
UV/Vis	Ultraviolet/Visible
wt%	Weight percentage
XRD	X-ray diffraction

Chapter 1

CHAPTER 1

1 DRUG DELIVERY AND POLY(2-OXAZOLINE)S

ABSTRACT The work described in this PhD thesis comprises the experiments and efforts on the synthesis, optimization and upscaling of defined high molecular weight poly(2-oxazoline)s in combination with their use in oral drug formulations. Although the work is highly interdisciplinary, it is written to obtain the degree of Doctor of Philosophy in Chemistry. The first chapter is meant to give a complete background on the work that has been performed over the four years of my PhD.

In the first section, the importance of drug delivery will be explained as well as the use of polymers in this growing field of drug development.

In the second section the focus will be put on oral drug formulations, as the main application within this PhD. Here a description is given about the type of formulations that exist, how to prepare them, how to characterize them and the state-of-the-art polymers used nowadays in oral drug delivery.

In the third part, poly(2-oxazoline)s are proposed as a suitable alternative to serve as an excipient in oral drug formulations. A short overview of 50 years of poly(2-oxazoline) work is described first with the focus on their monomer synthesis and an in-depth discussion on the cationic ring-opening polymerization mechanism towards poly(2-oxazoline)s. Furthermore, the different type of poly(2-oxazoline) homopolymers that are suitable for the application in drug delivery will be described, together with their thermal and solution properties. Finally, a short overview of biomedical applications of poly(2-oxazoline)s will be provided.

Chapter 1

1.1 HOW DRUG DELIVERY AND POLYMERS MEET

Development, formulation and delivery of drugs, medicines or active pharmaceutical ingredients (APIs) are omnipresent in our daily life. Enormous efforts are being done by companies, from big pharmaceutical multinationals to smaller drug development and formulation companies, to provide therapies for treatment of a broad scope of diseases. In this aspect a few steps are key, such as drug delivery and the corresponding formulations, and it is well-known that drug discovery and development takes a long time, *i.e.* approximately 15 years from discovery up to drug approval (**Figure 1**).[1,2] In this long-term development process, over 10,000 compounds can be screened in discovery to end up only with 1 approved drug after 15 years of work. With respect to retain more of these valuable drugs, and thereby increasing the ratio of approved drugs to screened compounds, a more detailed description of the development process (**Figure 2**) shows the importance of finding the right drug formulation. Inherently, the right drug formulation for a specific API should provide a good drug delivery and bioavailability of that API.[3–7]

According to the encyclopedia of medical concepts, the definition of drug delivery (systems) includes *´Systems for the delivery of drugs to target sites of pharmacological actions combined with the technologies employed to achieve this delivery, such as drug preparation or formulation, route of administration, site targeting, metabolism and toxicity.´* In this thesis we will mostly focus on improving oral drug formulations of highly water-soluble or poorly water-soluble model APIs, in order to optimize their drug delivery. Other important topics such as targeting, metabolism and toxicity will not be discussed.

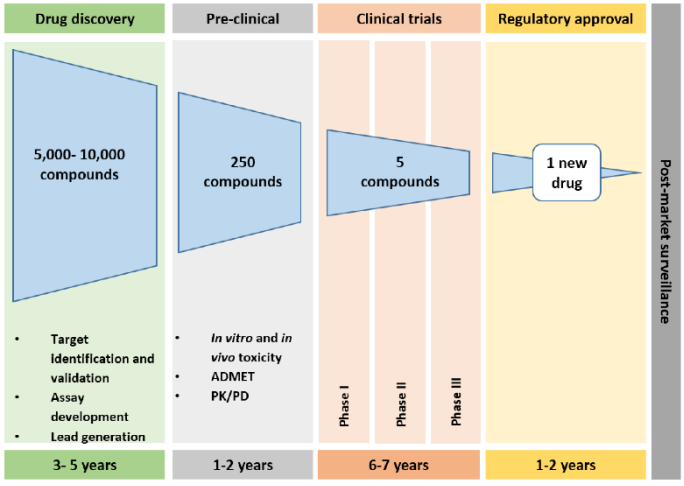


Figure 1. Drug discovery and development timeline over a period of approximately 15 years, starting from 10,000 lead compounds to one approved drug entering the market.[8]

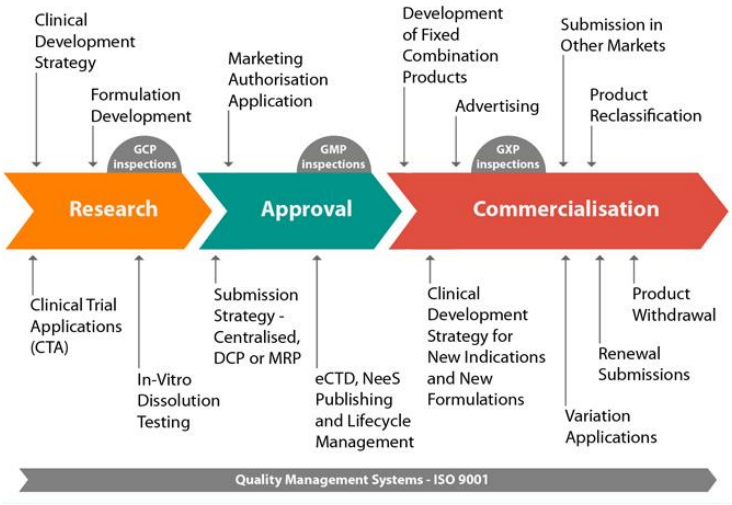


Figure 2. Detailed description of the drug development time line starting from research, *via* approval up to commercialization.[9]

A drug formulation generally consists of an API and aiding substances, or so-called pharmaceutical excipients or matrices. These excipients are pharmacologically not active, *i.e.* inert, and it can be seen as a generic name for every additive in a drug formulation, except for the API.[2,10,11]

In the case of small molecule drug delivery, the drug consists of relatively low molecular mass molecules (< 500 g/mol; analogous to the Lipinski rule[12] of five explaining the drug alikeness) which are regarded as ´active´ pharmaceutical ingredients. In contradiction to drugs, polymers or macromolecules are defined, as stated by IUPAC, as ´*A molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass*´[i], and can be regarded as an example of an excipient (**Figure 3**).

The conceptually different drug molecule and a polymer or macromolecule, are often united when drug delivery is regarded. The fact that a polymer is relatively high in molecular mass compared to an API, gives it properties like a glass transition, semi-crystalline behavior and tunable hydrophilicity making it an interesting carrier material for APIs or drugs. Within this field, in the last 10-20 years, the amount of scientific publications and reviews published on polymers

[i] ´In many cases, especially for synthetic polymers, a molecule can be regarded as having a high relative molecular mass if the addition or removal of one or a few of the units has a negligible effect on the molecular properties. This statement fails in the case of certain macromolecules for which the properties may be critically dependent on fine details of the molecular structure´ Source: PAC, 1996, 68, 2287 (Glossary of basic terms in polymer science (IUPAC Recommendations 1996)) on page 2289.

related to drug release increased exponentially (**Figure 4**).[13–15] According to IUPAC, a polymer drug is a ´polymer that contains either chemically-bound drug molecules or pharmacologically active moieties.´ Furthermore, polymers can also have a strong impact in physical mixtures with drugs. Here supramolecular interactions take place between the drug and the polymer, such as ionic interactions, hydrogen bonding interactions, *etc.* These interactions can play an important role in controlling the release of a certain API. There is also an increasing interest in oral drug formulations containing polymer carriers as excipient or aiding substance, for the APIs delivery, which is also the topic of this thesis.

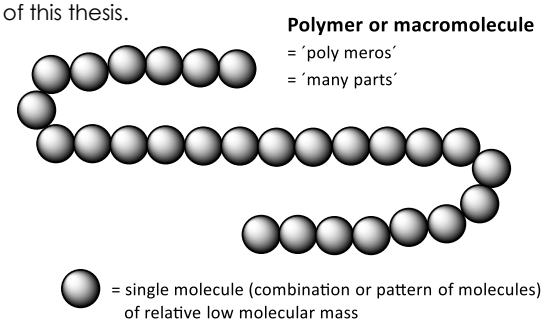


Figure 3. Schematic representation of a polymer or macromolecular structure, in its most simplified way according to IUPAC.

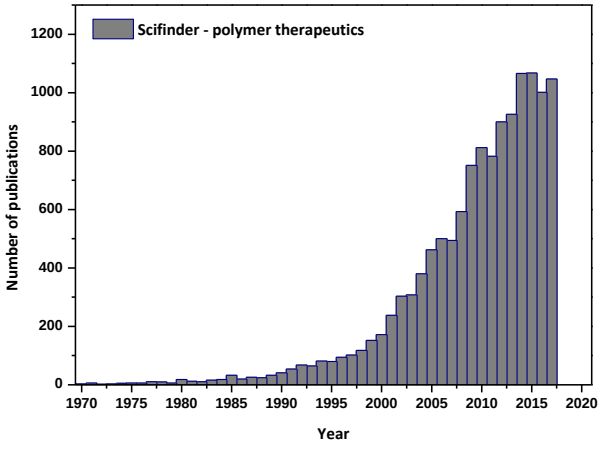


Figure 4. Visualization of the exponential increase of literature publications on polymer therapeutics, over the last 50 years, according to Scifinder.

Even though the fact that polymers and drug delivery go hand in hand, the following introduction and overview will be divided in two different parts, including a first part on oral drug formulations and a second part on the poly(2-oxazoline) polymer family. In the first part, the aim,

the advantages and the different types of oral drug formulations will be explained. In addition, a last section in this part will be devoted to the state-of-the-art polymers that are nowadays used as matrix excipient for oral drug formulations, combined with their current advantages, drawbacks and challenges. In a second part the poly(2-oxazoline)s will be introduced, starting from their monomers, polymerization mechanism and insights towards their interesting properties that have led to their application being strongly focused on the biomedical field.

1.2 ORAL DRUG FORMULATIONS

Drug formulation and delivery is essential to create efficient therapy for the treatment of diseases, *vide supra*. If one considers all different types of drug administration routes, including *e.g.* intravenous injections and transdermal, oral drug delivery is by far the most convenient and used method of administration, due to its high patient compliance, cost-effectiveness and ease of production, as explained in a review by Baghel *et al.* and many others (**Figure 5**).[16–21] In an article by Savjani *et al.*, it was reported that more than 85 % of the most sold drugs in the USA and Europe are orally administered drug formulations,[17,22] to name a specific literature example that supports the importance of oral drug formulations. Another review by Agrawal *et al.*[23] reports that more than 60 % of the small molecules on the market that are available as drugs, are administered orally.

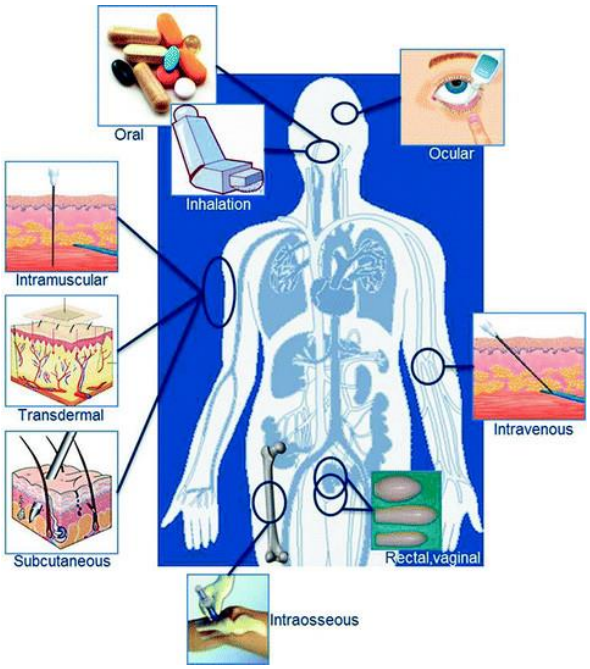


Figure 5. Overview of the different routes of administration for active pharmaceutical ingredients or drugs.[24]

The main challenge that is addressed by all these (oral) drug formulations is to increase the overall oral bioavailability, *i.e.* the amount of drug that reaches the blood stream (intravenous bioavailability is 100 %),[17,24,25] of the active pharmaceutical ingredient (API) or drug. For (oral) drug delivery, bioavailability[ii] is mostly related to two important aspects, *i.e.* the water-solubility and the permeability of the API, which is also how it was categorized in the 1990s, by Gordon Amidon and co-workers, as the **biopharmaceutics classification system** (BCS, **Figure 6**).[26,27] The development classification system (DCS), is an extension of the BCS based on more bio relevant media, such as fasted state simulated intestinal fluid (FaSSIF), to assess solubility and *in vivo* correlation. In the DCS, class II APIs are divided into class IIa, *i.e.* the dissolution rate limited APIs, and class IIb, *i.e.* the solubility limited APIs. In general, four different classes of drugs or APIs are classified, starting with class I that includes the highly water-soluble and highly permeable drugs are located, up to class IV that covers the poorly water-soluble APIs with low permeability through the membranes. In **Figure 6 (right graph)**, the boundaries of the different classes are defined, based on rather empirical calculations resulting from different *in vivo* studies.[28] If we consider this graph, an API is highly water-soluble if ´the highest dose strength of the drug which is administered, is soluble in less than 250 mL of water, over a pH range of 1-7.5.´ Furthermore, a drug is highly permeable when over 90 % of the drug is absorbed by the intestines in *in vivo* studies, *i.e.* possesses > 90 % bioavailability.

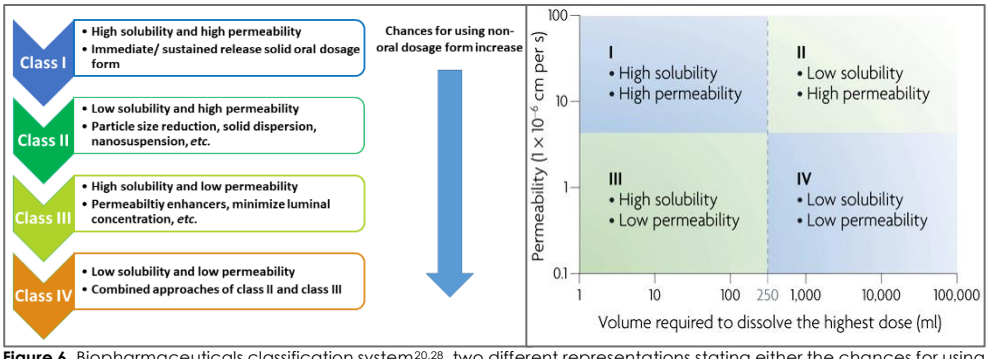


Figure 6. Biopharmaceuticals classification system[20,28], two different representations stating either the chances for using non-oral dosage forms along the different classes of drugs (**left**) or the ´boundaries´ between the different classes (**Right**).[20]

Important for all drug delivery systems is to keep the drug levels within the therapeutic window. The borders of this therapeutic window exist in the minimum effective level, *i.e.* resembling the minimum desired drug concentration in the plasma in order to have a therapeutic effect, and the maximum tolerating level or toxic level, *i.e.* above these

[ii] Bioavailability is defined as a measure of the rate and the extent to which a drug reaches the systematic circulation.

concentrations the drug concentration exceeds the desired levels in the blood and can cause side effects. In **Figure 7**, the desired controlled release level is indicated and most often drug concentrations vary in an alternating fashion around this profile upon drug administration. For very good water-soluble APIs drug concentration levels can increase very fast above the toxic level, but decrease very fast under the minimum effective level. If sustained release formulations are applied, burst release can be suppressed and drug levels can stay more continuous over time.[29] For poorly water-soluble APIs it is challenging to get to drug plasma concentrations that are in the therapeutic window. Therefore, the formulation should enhance the drug solubility.

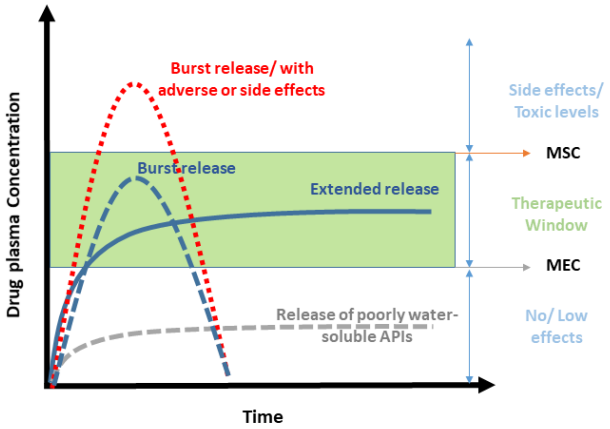


Figure 7. Schematic representation of the drug concentration in the blood versus time, with indication of the minimum effective concentration (MEC low) and the maximum safe concentration (MSC, *i.e.* toxic level). In between these levels, the therapeutic window is located to have desirable controlled release. Reprinted from[29]

Depending on the class of the API, different formulation strategies are necessary to increase the overall bioavailability of those drugs. It is a fact that the importance and chances for oral drug formulations decreases alongside the classes, with APIs from class IV bearing the highest chances of being administrated by non-oral dosage forms. Therefore, in the following explanation of enhancing drug bioavailability through oral drug delivery formulations, only the strategies for APIs of class I to III will be elaborated.

For the **highly water-soluble APIs of class I**, extended or **sustained release formulations (SRF, *vide* section 1.2.2)** are preferred to prolong their retention time in the gastrointestinal (GI) tract up to maximum 24 hours.[30] These SRF mainly consist of porous or swelling (cross-linked hydrophilic) matrices, which control or extend the release of the API, and protect the API from burst release in the different pH environments of the GI tract, upon administration of the tablets. In research studies comprising the investigation of these SRF, metoprolol tartrate (MPT), which is highly water-soluble and possesses a low melting temperature (T_m), is regarded as ´the´ model

compound to challenge release matrices for SRF as a function of drug load, *in vitro* and *in vivo* release studies, *etc.*[31–35]

If **class III APIs** that are highly water-solubility with poor permeability, are considered, also strategies concerning SRF are applicable (*vide* class I APIs). The poor permeability problems are often solved by combination of prolonged residence times in the GI tract and/or the use of permeation enhancing excipients, such as the use of prodrugs or tight junction openers. Metformin (MTF) hydrochloride can be regarded as one of the model APIs used to study the formulation effect of this class of APIs into further detail.[36–38]

Finally, the **class II APIs** (and in a certain extend also class IV) includes poorly water-soluble drugs with high permeability through the membranes, which resemble the majority of drugs developed in target and drug identification studies. The fact that this is the largest class of APIs in the BCS, is not remarkable, considering that the chemical composition of most drugs consist of highly (complex) structures with many hydrophobic units, *e.g.* aromatic moieties, rigid structures, *etc.*, and possess high tendencies to maintain complex crystalline structures; affecting and inducing most of the times poor water-solubility. Multiple strategies are found in literature to improve the water-solubility rate, and thus, overall bio-availability of class II APIs, such as **solid dispersions (SD, *vide* section 1.2.1)**, liquid dispersions, nano-milling or suspensions and cyclodextrin inclusion complexes. Since class II APIs are highly interesting for formulations, multiple model APIs, including *e.g.* fenofibrate, itraconazole and griseofulvin, have been studied over the years in order to challenge different types of excipients for the formulation of poorly water-soluble drugs. In the following sections only the solid dispersion strategy will be further elaborated, as it is most relevant for the application of poly(2-oxazoline)s (PAOx) as matrix excipient in different types of formulations with model APIs of class II.

In the following, **section 1.2.1** will explain in more detail the use of extended or SRF with some highly water-soluble model APIs from class I and class III. Furthermore, in **section 1.2.2** the solid dispersions for improving bioavailability of poorly water-soluble APIs from class II, will be explained. Regardless of the nature of the APIs, generalized methods for the preparation of those drug formulations exist, which are briefly discussed in **section 1.2.3**. Finally, in **section 1.2.4** currently marketed polymer excipients are described.

1.2.1 Sustained and/or controlled release formulations

If one considers controlled, sustained or extended release formulations, mostly polymer drug conjugates in which the drug molecules are covalently bound to the polymer are considered to tune the release of the drug upon an external trigger, *e.g.* pH, inside the human

body, if one considers extended release/circulation times upon intravenous injection.[24,39–44] Other types of carriers rely on the fact that the drug is encapsulated in a hydrogel, cross-linked network or hydrophobic polymer matrix, *via* supramolecular interactions between the drug and the carrier, in order to sustain the drug release.[45–47] In that way the release can be triggered upon external stimuli, by swelling of the matrix or by passive diffusion (**Figure 8**). In both covalent and non-covalent types of carrier systems, the interaction with the polymer is required for the controlled release of the drug as the bare drug possesses fast release kinetics in the body, which can induce too high drug levels in a short period of time. Apart from the swelling matrices or porous matrices, erosion based tablets also slow down the release of a water-soluble drug. Additionally, enteric coatings can prevent dissolution of a water-soluble drug depending on the pH, *e.g.* release is triggered by the higher pH upon entering the intestines and the drug is protected against the pH of the stomach. Most often, in contrast to the further mentioned SD, the highly water-soluble API is preferably present in the crystalline state. If the API would be present in the amorphous phase, the solubility rate would be even faster, which is unwanted in SRF.[48–51]

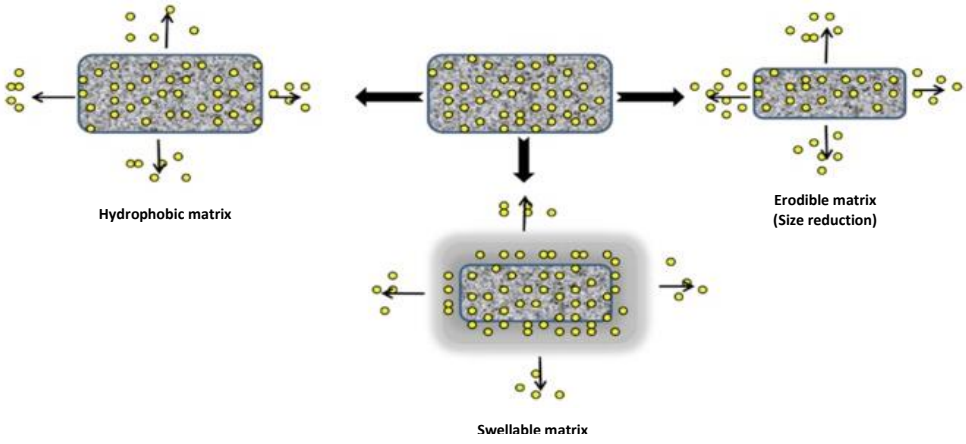


Figure 8. Illustration of the three most important sustained release formulation mechanisms, including swelling, erosion and diffusion (out of a hydrophobic matrix).[52]

1.2.2 *Solid dispersions*

The majority of new drugs in development (70 – 90 %) are being qualified under Class II and Class IV and are characterized by low aqueous solubility.[53,54] This number illustrates the importance to address these solubility issues and the fact that the pharmaceutical industry is continuously searching for new pharmaceutical excipients that can enhance dissolution rates and solubility of poorly-water soluble APIs. As such, these excipients could improve the success

rate for (newly) developed APIs, including APIs that are not yet formulated or not used because their further development is stopped due to above mentioned reasons. Over the last decade a number of strategies have been developed in order to enhance the bioavailability of poorly water-soluble drugs, including the use of surfactants,[55,56] cyclodextrin inclusion complexation,[17,57,58] emulsification processes,[56,59,60] co-solvency,[61] salt formation powder milling[62–65] and nanocrystals.[66–68] All of these techniques have been challenged with inherent problems concerning efficacy, product stability and versatility of the excipients and, therefore, only have specific uses. Moreover, to obtain more versatile/compatible and stable drug formulations, an increased amount of excipients must be added to the formulation in order to solubilize hydrophobic APIs, which increases the risk of side effects caused by the excipients, resulting in low patient compliance.[69,70]

Initially solid dispersions were introduced in 1960s by Sekiguchi and Obi. In recent years, the formulation of APIs in stable solid dispersions and solid solutions has received major interest to enhance bioavailability of poorly water-soluble drugs.[20, 56, 65,71–77] Improved bioavailability of drugs formulated in solid solutions or solid dispersions is generally based on the following two principles:

- Dispersion of amorphous API clusters in a binary demixed 2-phase system (amorphous solid dispersion), dissolving the API in in a crystalline (solid solution) or amorphous (glass solution) polymer matrix that enhances the availability of the API by keeping it in a higher energy state compared to the crystalline state (**Figure 9**).

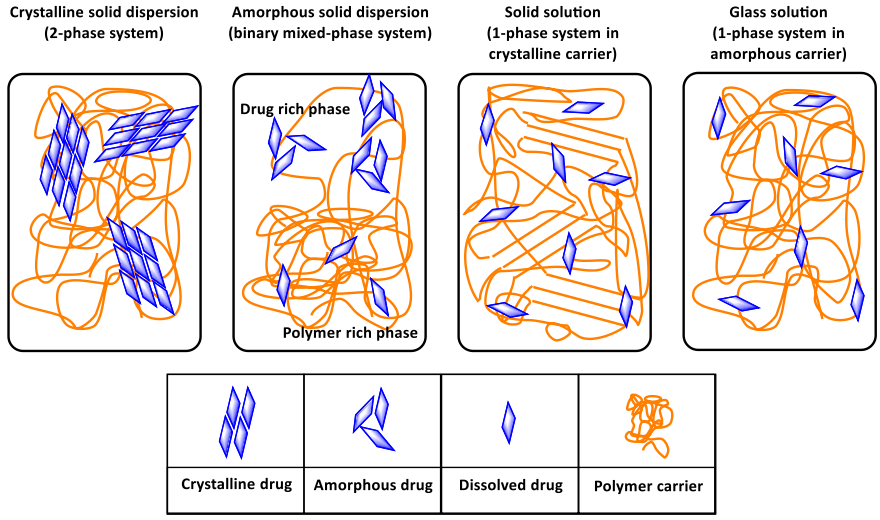


Figure 9. overview of the different drug forms in a polymer excipient, carrier or matrix: crystalline solid dispersions or sustained release formulations (**Left**), amorphous solid dispersions (**Middle**) and solid solutions (**Right**).[78]

• The polymer excipient should also interact with the API after release to stabilize a supersaturated solution state and prevent crystallization, which is also referred to as the spring-parachute principle (**Figure 10**).

Hence, the main task of solid dispersions and solid solutions is to prevent macroscopic crystallization of the API at all times, that is before and after release as well as during storage. The physical stabilization of the amorphous drug in solid dispersions results from strong polymer-drug interactions leading to an increased T_g of the polymer-drug mixture, compared to the T_g of the pure drug. As a result, the mobility of the drug is lowered, which inhibits nucleation and, thus, crystallization of the drug. Nucleation is also inhibited by the distance between separate drug molecules, (**Figure 9**, 2-phase versus 1-phase systems) especially in solid solutions, which is closely related to drug-polymer miscibility and drug content in the formulation. The success of SD and solid solutions is often related to the specific interactions between the drug and the polymeric carrier.

In general, solid dispersions can be prepared by precipitating from a solution of drug and carrier or by rapid cooling of a melt, referred to as the solvent method and melting (or fusion) method (*vide* **section 1.2.3-1.2.4**) for which spray drying and hot-melt extrusion/injection moulding (HME/IM) are the most important techniques, respectively.[72,79,80]

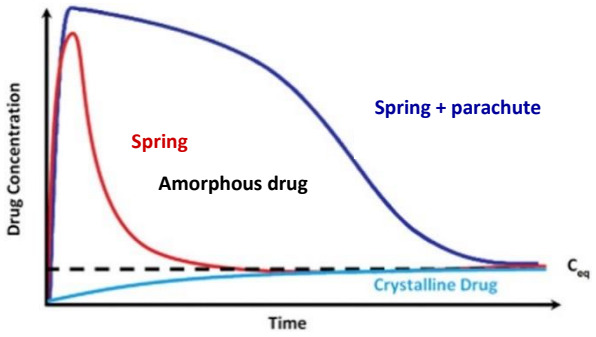


Figure 10. Drug concentration as a function of time for a crystalline drug and amorphous drug which is in a higher energy state (spring concept) and kept at a supersaturated state (spring + parachute concept).[20]

1.2.3 Methods of preparation

Solvent methods

In order to prepare SRF or SD, a solvent method can be applied. All solvent methods are based on the dissolution of both the API and the excipient in a common, preferably organic,

solvent, such as dichloromethane, chloroform, methanol, *etc.* In a second stage a drying step is applied, to get the oral drug formulation with the excipient in a dry and stable state. Examples of processes that use this basic technique of co-solubilizing the API with the excipient are solvent casting, spray drying and electrospinning. Even though solvent casting is the most basic technique, the slow evaporation of the solvent limits the formulation process as it provides more time and mobility for API crystallization. Often, this technique is used as a fast screening method for the preparation of SD, since this non-optimal drying technique can reveal insides about the ability of the excipient to keep the API in its amorphous form under worst-case-scenario conditions. Besides this initial screening by solvent casting, other more advanced solvent techniques can be applied to ensure fast evaporation of solvent. In **spray drying** (**Figure 11**), which is considered as the most used technique in industry, very small droplets are formed through an atomizer, which enlarges the surface to volume area to ensure quicker drying of the particles. Depending on the type of atomizer, such as pneumatic or rotary atomizers, the average size of the droplets can vary between 5 and 350 µm and will consequently determine the powder properties. Furthermore, a drying gas, mostly an inert gas such as nitrogen or argon gas, is introduced in the drying chamber to facilitate the drying of the formed particles.[81] Finally, the dried particles are collected and separated from the gas stream, most often by a cyclone, *i.e.* an accelerated flow which induces separation based on the density difference of the particles compared to the gas. In conclusion, spray drying can be performed from small to large scale and is dependent on a large variety of parameters, which makes it a complicated process, including the feed rate, inlet temperature, the flow of the gas particles, *etc.*; which can have an influence on the powder characteristics of the spray dried particles.[31,51–54] The requirements for the polymer and API, include good co-solubility in an organic solvent, ´good´ viscosity (not too high) to ensure drying of the droplets. Furthermore, the T_g of the polymer should preferably be higher than 50 °C, so that the polymer-API mixture can form a stable non-sticky powder. There is limited restriction regarding heat-sensitive APIs or APIs with extremely high T_m´s.[85]

Another solvent-based technique which can be used for drug formulation is **electrospinning** (**Figure 11**).[63,64,86,87] This technique has quite some similarities with the spray drying process, *e.g.* the feed contains a solvent in which the polymer and drug are co-solubilized, but there are also some important differences. Within the electrospinning process, a high voltage is applied on the material feed, instead of a heated gas stream. The fluid that comes out of a capillary or syringe pump is put under a large potential difference with the counter electrode, which creates charges and repulsion on the surface of the particles resulting in a jet and the formation of a so-called Taylor cone. The resulting jet is most often accelerated downstream the collector, while volatile organic solvents are quickly evaporated during the process. The

obtained powder-like fibers range from micrometer down to 100´s of nanometer size, depending on the set parameters, including feed ratio, concentration of the feed and applied voltage.

Solvent methods often give a powder formulation, meaning that a milling or granulation step is not necessary for downstream processing. Nevertheless, most often the powder has poor flowability and bulk density. After collection or compaction of the powder, a compression step is most often applied for getting the powder in its final dosage form.

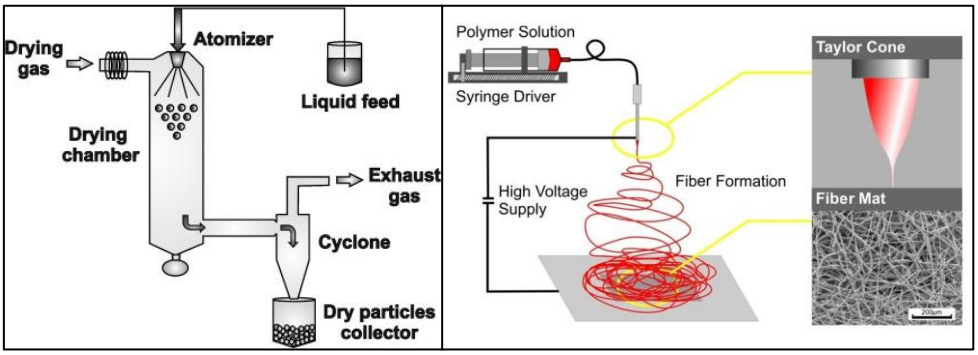


Figure 11. Schematic representation of a spray drying set-up[78] (**Left**) and lab scale set-up of the electrospinning process (**Right**).[88]

Melting methods

In contrast to the described solvent methods, the melting methods include formulation of the API and the polymer in absence of any (organic) solvent, under elevated processing temperatures and/or with the application of high pressures. The most important melting methods include HME, IM and melt granulation. Originally, **HME** was first introduced in the 1700s, for the processing of metal materials, whereas in the 1930s its use flourished with the increased production of polymeric bulk materials.[89] Nowadays, HME is still responsible for more than half of the processing techniques used in industry. However, for the pharmaceutical industry, HME is a relatively new technique, originating from the 1970s, when it was first applied for the preparation of oral drug formulations, by Doelker *et al*.[60, 85,90]

The HME process includes a feeding step, a conveying part including barrel and screw system for transport and mixing of the materials, a die and downstream processing equipment, such as grinders, millers and IM (**Figure 12**). In the feeding step the raw materials, including API and polymer, are added to the barrel. The screw system in the heated barrel, which can be single or twin-screws (co or counter-rotating), moves the heated material with a certain force or

torque towards the die. In order to melt-mix the polymer with the API efficiently, preferably twin-screw extruders or compounders are applied. The dimensions of the barrel and the screw, *i.e.* determined by the length to diameter ratio (L/D), will be one of the important parameters to achieve complete mixing during the process. Additional parameters that can be varied are rotation speed of the screws and the temperature of the barrel, preferably below the degradation temperature of the API and above the T_g (or T_m) of the polymer.

There are certain advantages of HME compared to spray drying. Firstly, the simplicity of manufacturing on an industrial scale makes it popular with pharmaceutical manufacturers. It has also been shown that more uniform (molecular) dispersions are formed with HME resulting in enhanced bioavailability.[60] The absence of residual solvents in the produced formulations is preferable, although the different types of polymers that are suitable for HME are more limited compared to the ones that can be applied in spray drying. On the contrary, high processing temperatures and shear magnitudes, applied in HME, can be a drawback in terms of degradation of the polymer excipient and API. Another significant drawback using this technique is that HME often requires an additional processing step such as grinding, milling, calandering and cutting of extrudates for the production of, e.g. tablets, films, pellets or mini-tablets. Nevertheless, these additional processing steps, *e.g.* in HME coupled to IM, permits manufacturing of very complex shapes and allows to accurately control the three-dimensional structure of the processed materials.[91]

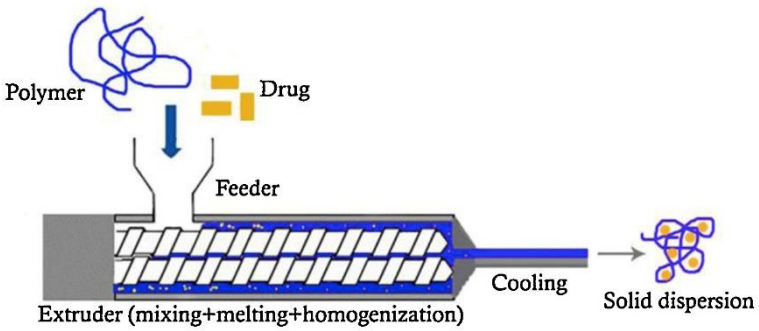


Figure 12. Schematic view of a hot-melt extruder including the feed of the API and polymer, twin-screw set-up and barrel with cylindrical die.[85]

Besides milling and grinding of the extrudates, **IM** (**Figure 13**) is very often coupled as downstream process equipment to ´shape´ the extrudates in the preferred manner. In fact, the IM process closely resembles the HME process without the mixing and homogenization of the API and polymer mixture. Generally, an injection moulder consists of an axial screw in a barrel, a

plunger and a cavity or mould. The plunger shapes the extruded material in a predefined and closed mould under high pressure and certain temperature conditions. Upon processing the pressure and temperature will be changed from high to low, to end up with a cooled tablet that can be withdrawn from the mould as final dosage form. The different process temperatures and pressures can be varied according to the nature of the API and polymer melt, to optimize the process with regard to polymer T_g, pressure resistance and plastic recovery of the applied material.

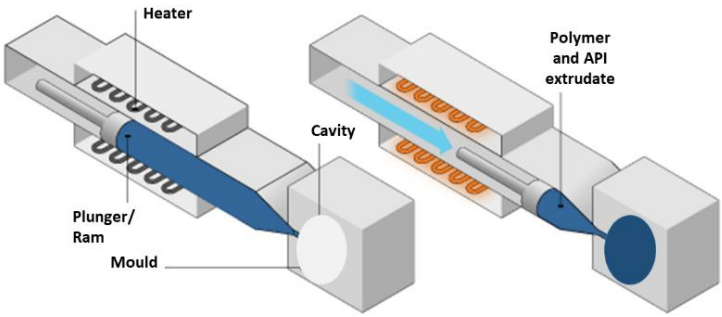


Figure 13. Simplified representation of an injection moulder with plunger and tablet mould, that can be coupled to a hot-melt extruder.[92,93]

In **Table 1**, an overview is given of the main advantages and disadvantages that are correlated to solvent and melting formulation methods.

Table 1. Advantages and disadvantages of spray drying *versus* hot-melt extrusion for the preparation of SD or SRF; summarized from references.[60, 74, 79,85]

Solvent Method (spray drying)	Melting Method (HME)
No problems regarding thermal degradation of API and/or polymer	Thermal degradation can occur
Large range of polymers applicable	Limited range of polymers applicable
(Organic) solvent needed Common solvent for API and excipient required	No solvent
Large scale applicable/ often batch process	Continuous processing on large scale easy and straightforward
Downstream processing: poor flowability/low bulk density	Downstream processing can be challenging and needed: *e.g.* milling, IM

1.2.4 Characterization methods for oral drug formulations

Analysis and spectroscopy methods

Characterization of the retrieved oral drug formulations can be done using several techniques including thermal characterization, powder analysis and microscopy. For thermal characterization of the formulations, thermogravimetric analysis (TGA) is often used to retrieve information concerning thermal degradation (prior to processing) and residual moisture content of the prepared formulations. Furthermore, differential scanning calorimetry (DSC) is used to gather knowledge about the glass transition temperature and melting temperature of the polymer, the API and their (physical) mixture (**Figure 14**). The T_g will be influenced by interaction of the API with the polymer matrix in a SD or SRF. If the API acts more like a filler, *i.e.* filling up empty spaces between the polymer chains, or strongly interacts with the polymer this will lead to an increase in the T_g. Poor interactions between the API and the polymer may result in a plasticizing effect, or even separate thermal transitions may be observed when phase separation occurs. A physical mixture (*i.e.* non-formulated) of the API and polymer will also show two distinctive T_g´s (the T_g of the drug is often below zero). Finally, the quantitative and qualitative analysis of the APIs crystalline fraction can be determined by DSC.[94–96]

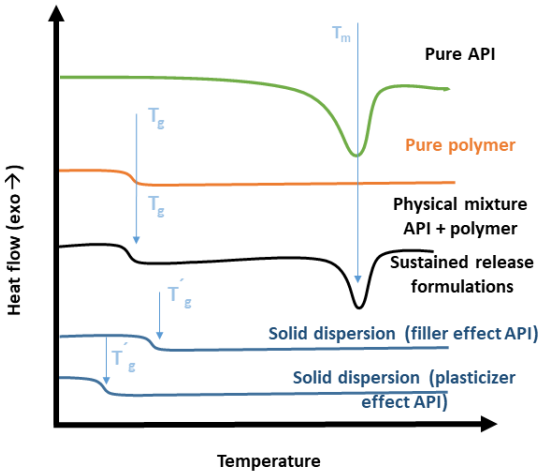


Figure 14. Example of a differential scanning calorimetry (DSC) diagram, including the different T_g´s and melting peaks (T_m) for the pure API and polymer, a physical mixture of both and a solid dispersion or sustained release formulation.

Alternatively, *via* powder X-ray diffraction (XRD, **Figure 15**), one can look at the crystalline fragments (indicated by sharp peaks in an the XRD spectrum) of the API compared to the amorphous fractions of the polymer (broad bands in XRD spectrum), to qualitatively

determine the interaction between the API and the polymer in a drug formulation. It is also possible to determine quantitatively the crystalline fraction, nevertheless it is not that straightforward if polymer-API mixtures are considered.

Other characterization techniques to identify polymer-drug interactions include infrared (IR) and Raman spectroscopy or mapping.[97] This type of spectroscopy, irradiates the sample with a laser while a scattering pattern is observed. Similar to XRD, smaller molecules or APIs are giving more defined scattering patterns compared to polymers, giving IR and Raman spectroscopy the ability to detect small fragments of crystalline drug in the amorphous drug formulations with high sensitivity (< 1 % mass of tablet). An excellent review from Palermo *et al.* summarizes all of these techniques described above.[98]

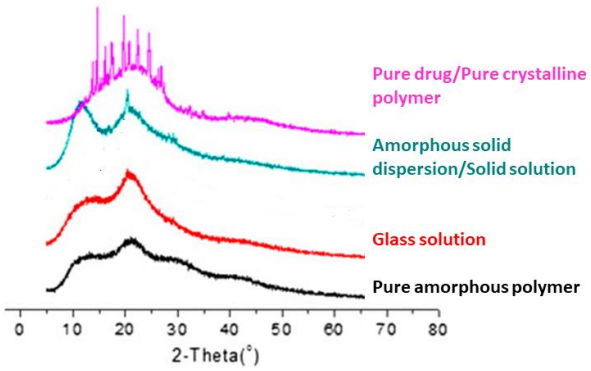


Figure 15. Example of an X-ray diffraction diagram and proposed peak shapes for the pure drug, polymer, solid dispersion and a solid solution.

Dissolution testing or kinetics

An important part in the characterization of oral drug formulations comprises the release of the drug as a function of time in a dissolution medium, *i.e. in vitro* dissolution testing, performed for quality control purposes or to predict *in vivo* behaviour.[54, 94,99,100] It should be noted that all of these test are standardized according the pharmacopeia and regulation agencies. The release kinetics of the drug from the (polymer) matrix are tested in *in vitro* dissolution media, being at first aqueous buffer media with pH ranges of 1.2-7.5. Secondly, biological relevant media should be introduced, combining buffer and enzymes in order to get better results according to *in vitro-in vivo* correlations, referred to as simulated gastric fluid (SIG, pH 1.2) and simulated intestinal fluid (SIF, pH 6.5). The temperature of the dissolution tests is most commonly set at human body temperature, 37.5 °C. In order to maintain poorly water-soluble drugs soluble

in the dissolution medium, most dissolution tests are performed in ´sink conditions´. Sink conditions are defined as ´the volume that exceeds at least three times the volume that is needed to achieve a saturated solution of the API´.[99] Despite these sink conditions, there are still a number of drugs that have such poor solubility, that another medium such as, isopropanol or other aqueous alcoholic media, has to be used to carry out dissolution tests. The analysis of the released drug is most often performed by UV/VIS spectrophotometric analysis or high-performance liquid chromatography (HPLC) methodologies.

A final step could include the performance of bio-relevant tests in animals or humans (last stage), *i.e.* measuring plasma concentration in time after oral administration of the prepared oral drug formulations. Most often *in vitro-in vivo* correlations are not straightforward since predictions often fail due to the general complexness of *in vivo* studies. In conclusion, drug dissolution tests of the designed drug formulations will have to be performed in *in vivo* assays in order to finally reach the market. More details and discussion about *in vivo* dissolution tests and different important parameters can be found in a literature review by Newman *et al.*[73]

Shelf-life stability

The stability of the drug formulations in time, mostly regarded to SD, has to be assessed. If for example a SD is characterized to possess only amorphous fractions of the API, thereby improving the bioavailability, the question will rise how long this dispersion is stable (remains amorphous) over a certain period of time. Mainly, these tests include the performance of (a few of the) aforementioned characterization techniques at different times after preparation of the specific drug formulations. On the one hand the characterization can take place after the formulations have been stored at the ideal storage conditions for a time period of *e.g.* 6-24 months. On the other hand, standardized tests describe stability essays at elevated temperatures and humidity that simulates the long-term stabilization of the formulation, in order to drastically shorten the time for stability testing.[101,102]

1.2.5 State-of-the-art polymers as matrix excipients: limitations and challenges

Various polymeric materials (**Figure 16, Table 2**), such as poly((methyl) methacrylate)s (P(M)MA) and amino copolymers thereof;[103] poly(vinyl pyrrolidone)s (PVP) and copolymers with *e.g.* vinyl acetate;[66,104–106] poly(ethylene glycol-co-vinyl acetate-co-vinyl caprolactam) graft co-polymers (PEG-VA-CL)[107], hydroxypropylmethylcellulose (HPMC),[33, 64,80,81] thermoplastic polyurethanes (PU),[48] *etc.* have been used for the production of solid dispersions and/or sustained release formulations.

Despite the increasing potential of the use of polymers as excipient in formulations, there is still a limited number of polymer-based formulations available on the market, today. This number accounts to approximately 24 formulations based on the solid dispersion principle which have been marketed over the last 30 years, which are clearly summarized in a recent review by Jermain *et al*.[108] The main reason can be found in the current drawbacks associated with these marketed polymer excipients, such as the lack of physical and chemical stability, processability, solubilizing capability, long-term shelf-life stability and versatility (structurally). In addition, none of the aforementioned polymers could serve as a platform for the formulation of a wide range of APIs.[56,68,69] **Table 3**, gives an overview of the current limitations and challenges for the commercial polymer excipients.

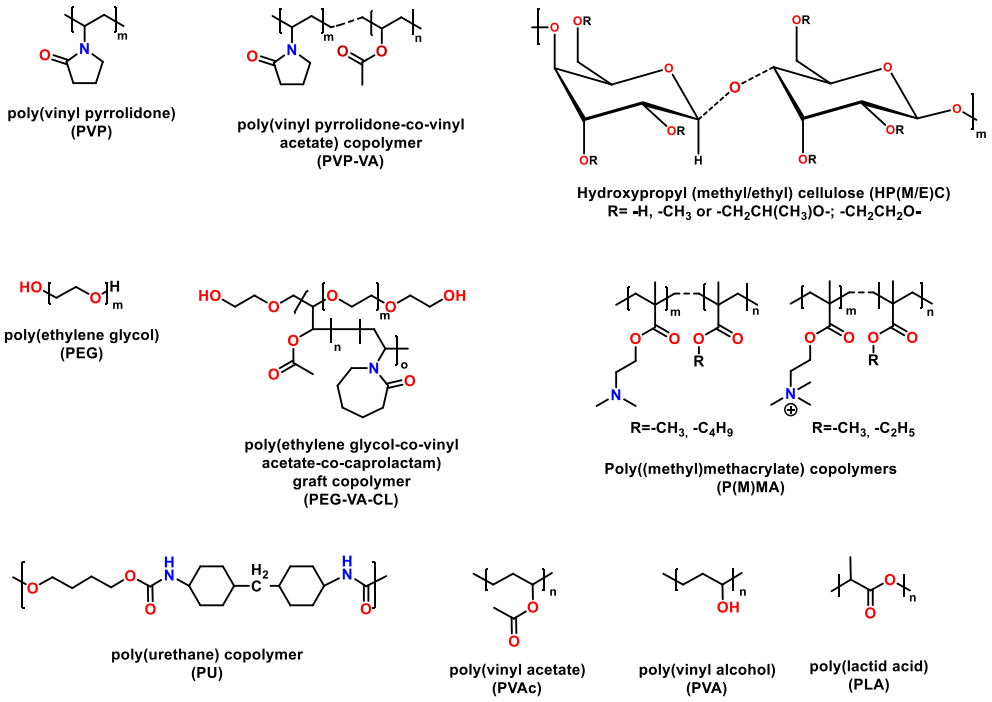


Figure 16. Overview of the chemical structures of the polymeric excipients used in solid dispersions and sustained release formulations.

Table 2. Overview properties various (non-)marketed (co)polymers for solid dispersions or sustained release formulations.[65]

Polymer excipient	Tradename(s)	Thermal properties (T_g, °C)	Method of preparation	Immediate (IR) or sustained release (SR)
PVP	Povidone®	168	Hot-melt extrusion/ spray drying	IR
PVP-VA	Plasdone®	106	Hot-melt extrusion/spray drying	IR
HPMC	Methocel®	170-180	Spray drying/ direct compression	SR/IR
PEG-VA-CL	Soluplus®	70	Spray drying	IR
P(M)MA	Eudragit®	130	Hot-melt extrusion/ spray drying	IR/SR
Amino PMA copolymers	Eudragit® E	56	Hot-melt extrusion	IR
PVAc/PVAL	Parteck®	40-80	Spray drying	SR/IR
PU	Tecoflex®	-26-10 (T_m 50-80)	Hot-melt extrusion	SR
PLA	Expansorb®	60 (T_m 130-180)	Microcapsules	SR
HEC	Natrosol®	120	Hot-melt extrusion/IM	SR

Table 3. Overview of the main limitations, challenges and commercial availability of P(M)MA, PVP, PVP-VA, PEG-VA-CL and HMPC as matrix excipient for API formulations.

Polymer excipient	Main limitations and challenges
P(M)MA	• Degrades above 155 °C limiting applicability for HME • Has been shown to initiate decomposition of the API • Low T_g can limit physical stability (amino methacrylate polymer, T_g 56 °C) • No commercial formulations marketed; yet FDA approved
PVP	• Limited physical stability because of its hygroscopic nature • High T_g: need plasticizing API or plasticizer if processed by HME • Residual peroxides, Low thermal stability • Commercial formulations marketed
PVP-VA copolymer	• Relatively hydrophobic: limits formulation of poorly water-soluble APIs • HME possible for APIs with T_m above 150 °C • Commercial formulations marketed
PEG-VA-CL	• Limited physical stability due to potential hydrolysis of vinyl acetate groups • Low Tg can limit physical stability of solid dispersion • No commercial formulations marketed; yet FDA approved

HPMC	• High T_g: not very suitable for HME • Used in spray drying and direct compression • Difficult to mill • Limited solubilizing capability (max 40 % in sustained release formulations) • Not relevant for thermally unstable APIs • Commercial formulations marketed

In the context of these limitations of commercial polymer excipients, PAOx polymers are proposed as an alternative for the preparation of oral drug formulations. Apart from their proven biocompatibility, the use of PAOx can offer advantages considering tunability of the overall hydrophilicity and thermal properties, processability and high physical and chemically stability.[109,110] The structure of PAOx is amphiphilic and contains a tertiary amide group in the repeating unit, which are proposed to be beneficial for its use as excipients. An overview of their synthesis, properties and application will be elaborated in the following section.

1.3 POLY(2-OXAZOLINE)S

Numerous 2-oxazolines have been used in the cationic ring-opening polymerization (CROP) to obtain the corresponding poly(2-oxazoline)s (PAOx, also referred to as POx, POz). PAOx originate from 1966, when they were reported for the first time by four independent research groups.[111–114] Besides their use as monomers, 2-oxazolines are also commonly used as ligands in asymmetric catalysis.[115–117] In the last decade PAOx have received increasing interest, especially for biomedical applications. Several reviews on this topic have recently been published providing an excellent overview of the current state-of-the-art.[109,118–123] Even though the application potential of PAOx is tremendous and has been the focus of many review articles, it is relatively difficult to get a complete overview of the different PAOx structures that have been reported as well as a systematic insight in the properties of these different PAOx.

In **section 1.3.1**, first a brief description of the 2-oxazoline monomer synthesis is given. Furthermore, the CROP of 2-oxazolines will be described (**1.3.2**) including the most important side reactions (**1.3.3**). Finally, a comprehensive overview of PAOx homopolymer structures (**1.3.4**) and their thermal (**1.3.5**) and solution properties (**1.3.6**) will be elaborated. In section **1.3.7**, PAOx and their most important biomedical applications are discussed.

1.3.1 Synthesis of 2-oxazoline monomers

2-Substituted 4,5–dihydrooxazoles, *i.e.* 2-substituted-2-oxazolines, referred to as 2-oxazolines in the following paragraphs, are the most commonly used cyclic imino ether monomers in the cationic ring-opening polymerization (CROP). In addition some 4- and 5-substituted 2-oxazolines have been polymerized by CROP.[124–127] Typically, 2-oxazolines are synthesized *via* the direct synthesis from non-activated carboxylic acids,[128] the Witte-Seeliger synthesis from nitriles[129,130] or the Wenker method,[131] *i.e.* a two-step synthesis *via* cyclization of β-halo amides (see **Figure 17**).

Other less common synthesis routes, *e.g.* alpha-deprotonation of 2-methyl-2-oxazoline followed by alkylation towards more complex 2-oxazolines, are also reported.[132,133] For a more detailed overview and description of the synthesis of 2-oxazolines one can refer to the recent review by Verbraeken *et al.*[134]

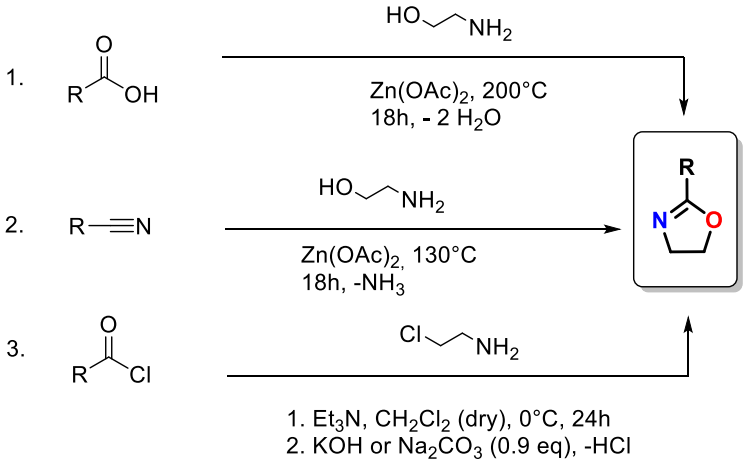


Figure 17. Overview of the synthesis of 2-oxazolines monomers. 1. Direct synthesis *via* non-activated carboxylic acids, 2. The Witte-Seeliger method starting from nitriles and 3. The (modified) Wenker method. For reaction 1 and 2, any zinc salt can serve as catalyst for the reaction.

1.3.2 Cationic ring-opening polymerization of 2-oxazolines

The first CROP of 2-oxazolines was reported 50 years ago by four independent research groups, resulting in a new class of polymers, *i.e.* the PAOx.[111–114] The CROP mechanism consists of three steps, initiation, propagation and termination (**Figure 18**).

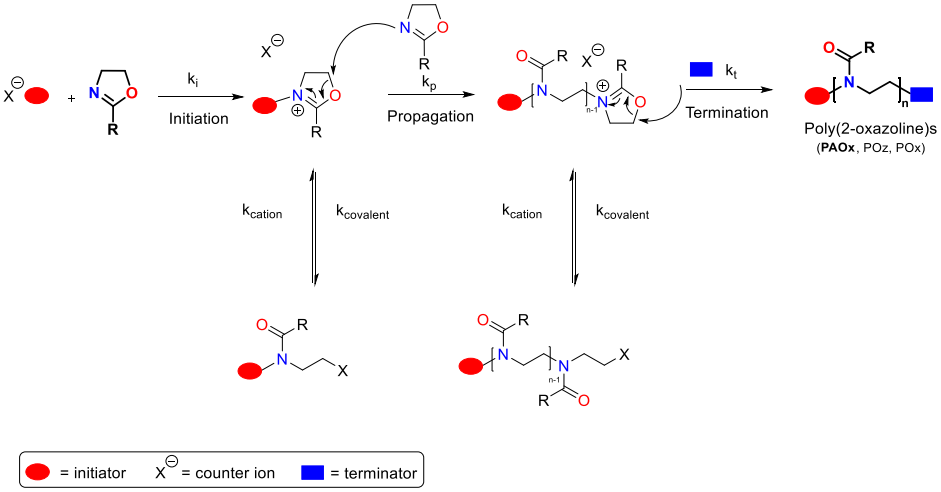


Figure 18. The cationic ring-opening polymerization of 2-oxazolines under ideal conditions (no side-reactions included), three-step mechanism with initiation, propagation and termination step. Depending on the used initiator and counter ion, the ring-opened version of the oxazolinium species after initiation and during propagation are shown.

Initiation

In the first step of the CROP a nucleophilic attack of the nitrogen lone pair of the 2-oxazoline monomer onto an electrophilic initiator forms an oxazolinium cation, initiating the polymerization reaction. Different initiator systems can be used including alkyl sulfonates such as methyl *p*-toluenesulfonate (MeOTs), which is most frequently found in literature, *p*-nitrobenzenesulfonates (nosylates) and trifluoromethanesulfonates (triflates), alkyl, benzyl and acetyl halides, oxazolinium salts (synthesis *vide* **chapter 4**) and lewis acids.[135–143] It is important to note that the counter-ion of the initiator influences the equilibrium between the cationic oxazolinium species *versus* the ring-opened isomer or covalent species. It is reported that the ratio of cationic *versus* covalent species is greatly dependent on the leaving group properties of the counter-ion, and influences the propagation (*vide infra*).[141,144] Moreover, the first monomer addition often leads to the covalent species while the cationic oxazolinium is formed upon further monomer addition, which is influenced by interactions with the carbonyl group of the neighboring penultimate ring-opened poly(2-oxazoline) unit. Also functional initiators can be used as long as they don't bear any nucleophilic character that could interfere with the CROP. An overview of various functional initiators that have been reported can be found in a review by Lapinte *et al.*[145]

Propagation

Subsequently, in the propagation step of the CROP the 2-oxazoline monomer attacks the cationic oxazolinium intermediate, forming the poly(2-oxazoline) backbone by ring-opening and the formation of an amide while retaining the living oxazolinium chain-end.[146] Also here the equilibrium between cationic and covalent species plays an important role, as propagation proceeds much more rapidly on the cationic species ensures the propagation reaction. It has been reported that the initiator counter-ion and its ability to form more cationic species, *e.g.* with bromides, iodides, tosylates or triflates, leads to a faster propagation rate constant (k_p) as polymerization proceeds nearly exclusively on the cationic species when both cationic and covalent propagating species are present.[141,144] In an ideal polymerization no chain coupling, transfer or termination reactions occur resulting in the living CROP of 2-oxazolines. The livingness of the polymerization results in control over molecular weight and narrow molar mass distribution, *i.e.* dispersity (Đ), if the initiation is fast and quantitative. Therefore, often the propagation rate constant is taken as the overall polymerization rate constant for the living CROP of 2-oxazolines. In the absence of irreversible termination, the rate of propagation follows first order kinetics according to the following formula (1) which can be integrated as formula (2).[147,148]

$$-\frac{d[M]}{d(t)} = k_p\,[P^+][M] \qquad (1)$$

$$ln\left(\frac{[M_0]}{[M_t]}\right) = k_p\,[I_0]\,t \qquad (2)\ ^{iii}$$

Unfortunately chain transfer reactions, such as β-elimination, and coupling reactions have been reported to be inherent to the polymerization, which become especially important when aiming for high molar mass PAOx.[149–153] A more detailed overview of the CROP mechanism and chain transfer mechanism can be found in a recent review by Verbraeken *et al*. and in **section 1.3.3** of this chapter.[154] It is important to note that, the synthesis of well-defined PAOx with a narrow molar mass distribution requires thorough purification of the initiator, monomer and solvent used for the polymerization, to avoid the presence of nucleophilic impurities, including traces of moisture, that induce chain transfer and termination reactions. A recent patent, by Monnery *et al.*, shows the ability of synthesizing defined high molar mass poly(2-oxazoline)s by excluding these side reactions under carefully optimized reaction conditions, including specialized vacuum techniques and low temperature polymerization (*vide* **chapter 4**).[155]

Termination

Finally, termination of the CROP of 2-oxazolines occurs *via* nucleophilic attack of an added termination agent on the living cationic chain-end. Most strong nucleophiles, such as methanolic potassium hydroxide, carboxylates and amines, can be used to terminate the living oxazolinium polymer chain end onto the 5-position of the activated 2-oxazoline ring (**Figure 19**). Termination on the 2-position – where the side-chain is located – has also been reported for other, weaker nucleophiles, particularly water as most important example.[156,157]

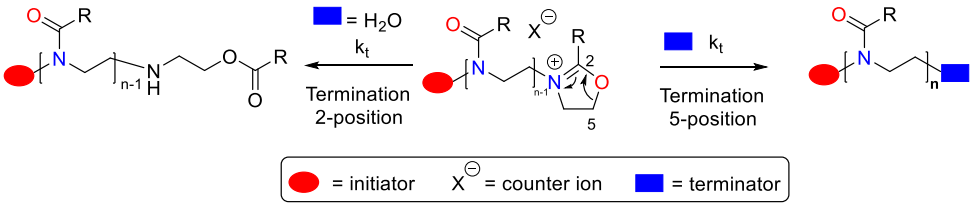


Figure 19. Example of termination reaction respectively on the 2-position, *e.g.* with water, and on the 5-position of the oxazolinium chain-end.

[iii] The symbols represent the initial monomer concentration $[M]_0$, monomer concentration at certain time point $[M]_t$, the concentration of propagating species $[P^+]$ assumed to be equal to the initial initiator concentration $[I]_0$ for an ideal CROP, time and propagation rate constant k_p.

With the CROP being a living/controlled polymerization mechanism, functionalities can be easily introduced during initiation and termination with high end-group fidelity. Oxygen, nitrogen, sulfur and carbon centered nucleophiles, which exclusively terminate via reaction at the 5-position, can be used to introduce functional groups at the ω-chain-end. A near complete overview can be found in a recent review by Glassner and Vergaelen *et al*.[158]

1.3.3 Insights in side reactions of the CROP of 2-oxazolines

Most of the important findings on side reactions during the CROP of 2-oxazolines were done in the early years around 1960-80s, by the groups of Levy, Litt *et al.* and Greenhalghs *et al.*,[149–151,159] who were the first in describing the chain transfer and coupling processes induced by impurities such as water and interfering polymerization solvents or nucleophilic side-chains. Follow-up research studies on this topic were again initialized in the years 2000 by Diab *et al.* and Park and Kataoka *et al.*, [160–163] together with the revival of the use of PAOx polymers in all kinds of primarily biomedical applications.[109, 118,164,165]

Most of the side reactions described in **section 1.3.2** are induced by **extrinsic contaminants**, including water, ammonia and other nucleophiles, of which the reaction mechanisms have been described by Greenhalghs *et al*.[159] and Monnery *et al.*[153] In order to deal with these extrinsic impurities, stringent purification methods can be applied,[166] following an *in situ* purification of the reaction mixture, *via* the sacrificial initiator method (*vide* chapter 4) and high vacuum techniques (analogous to anionic polymerization purification processes) as described by a patent to uniformly synthesize PAOx polymers, by Monnery and Hoogenboom.[155]

Intrinsic side reactions

The most difficult impurities to deal with are the **intrinsic side reactions** induced by components of the polymerization reaction mixture, including the monomer, solvent, initiator and temperature. Litt *et al.* already identified the chain transfer processes that are independent of the purification methods used, indicating that they are intrinsic side reactions for the CROP of 2-oxazolines. In this regard, chain transfer to monomer can occur (**Figure 20**) without any impurity being present in the polymerization mixture. The origin of this chain transfer to monomer reaction can be found in the ability of the 2-oxazoline to act as a base instead of a nucleophilic monomer for the CROP of 2-oxazolines. *Via* this route, the 2-oxazoline base induces β-elimination, when a proton is abstracted from the living propagating oxazolinium species, resulting in a ´non-living´ enamine species. (**Figure 20**, top) Subsequently, the ´non-living´ enamine polymer can act as a nucleophile attacking another living oxazolinium polymer chain, described as chain coupling

(**Figure 20**, bottom). However, due to the lower reactivity of the enamine compared to the 2-oxazoline monomer, chain coupling mostly occurs at higher monomer conversion.

Analogous to this intrinsic chain transfer reaction mechanism, chain transfer to solvent and chain transfer induced by extrinsic impurities (*vide supra*) can occur inducing either chain coupling, branching or termination of living species in the polymerization mixtures. All of these side reactions attribute to broadening of the mass distribution and higher Đ, thus non-uniformity of the PAOx polymers synthesized. It has been reported that lowering the reaction temperature, around 42 °C, and aiming for lower monomer conversions helps decreasing the amount of chain transfer and coupling reactions. [160–163,167] In addition, it is found that chain transfer occurs less when the β-position is more substituted. It is however still unclear why chain coupling reactions also happen for *e.g.* 2-phenyl-2-oxazoline (PhOx), which does not have a β proton available to undergo elimination. Until today, it is not clear whether other side reactions may be occurring too, which may explain the observed coupling in the polymerization of PhOx and analogous monomers.

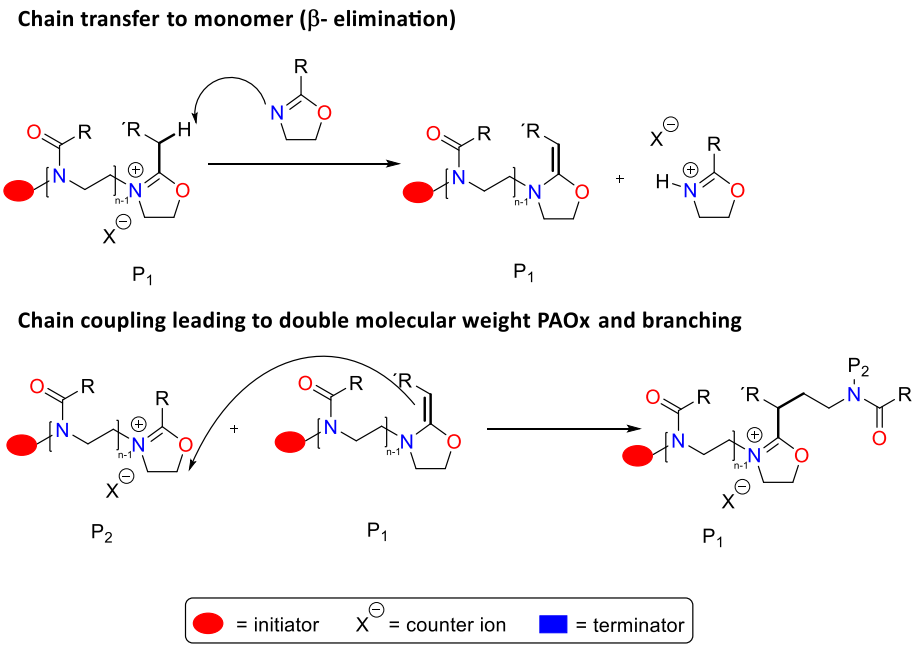


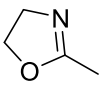
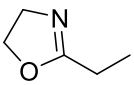
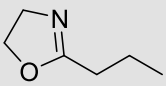
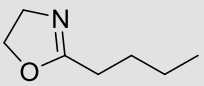
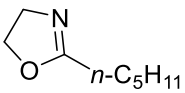
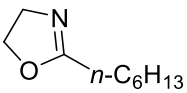
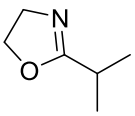
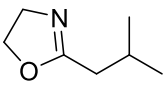
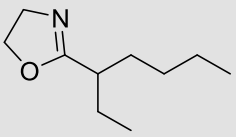
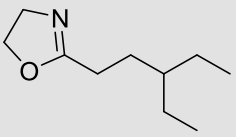
Figure 20. Schematic overview of the chain transfer to monomer, as in β-elimination, that occurs as an intrinsic side reaction during the propagation step of the CROP of 2-oxazolines. (**Top**) Chain coupling reaction resulting from the formation of the enamine non-living polymer; which can act as a nucleophile to propagate other living oxazolinium chain-ends. (**Bottom**) (Reformat from ref.[153])

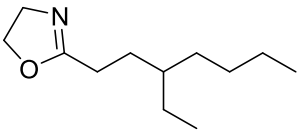
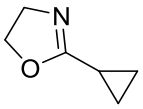
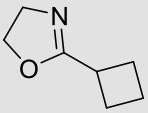
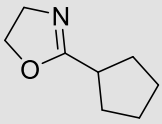
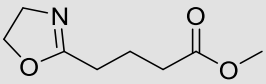
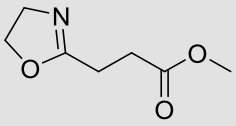
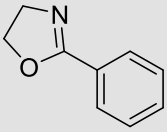
In the last years the group of Hoogenboom *et al.* reported the synthesis of defined PAOx polymers up to 200,000 g/mol indicating that over the years the tremendous efforts in understanding the mechanistic details of the side reactions for the CROP of 2-oxazolines enabled suppression of these intrinsic side reactions resulting in a reproducible process to synthesize defined PAOx polymers with a MW over 20,000 g/mol.[168,169] This important pioneering work serves also as basis for this PhD project.

1.3.4 Poly(2-oxazoline) homopolymers

In the last 50 years an enormous range (over 100) of PAOx with varying side-chain structures has been reported in the literature. In **Table 4,** the most important PAOx homopolymers for this PhD project were selected from the comprehensive review by Glassner, Vergaelen and Hoogenboom, which reports all 2-oxazoline monomers whose respective PAOx homopolymers have been prepared by CROP, so far.[158]

Table 4. Condensed version of all the tables found in the comprehensive review of Glassner *et al.*;[158] selection is made according to the homo PAOx polymers of possible interest for this thesis.

Monomer	Abbr.	Ref.	Monomer	Abbr.	Ref.
Linear poly(2-alkyl-2-oxazoline)s					
	MeOx	113,170–173		EtOx	113,170–177
	*n*PrOx	162, 170, 172,177–181		*n*BuOx	170,172
	*n*PentOx	170,172		*n*HexOx	172,182
Branched poly(2-alkyl-2-oxazoline)s					
	*i*PrOx	161–163, 178,183–186		*i*BuOx	187–189
	EPOx	190		3EPOx	190

	EHOx	189		cPrOx	178,191,192
	cBuOx	193		cPentOx	193
Functional poly(2-oxazoline)s					
	C3MestOx	194,195		C2MestOx	150,194–196
Poly(2-aryl-2-oxazoline)s					
	PhOx	113, 128, 171, 173,197–201			

The simplest and most widely studied PAOx, with regard to systematic investigations of the CROP, are those with linear or branched alkyl side-chains (references see **Table 4**). The propagation rate constants (k_p) (overview in **Table 5**), assuming 100 % initiator efficiency for *e.g.* MeOTs, of 2-alkyl-2-oxazolines decrease generally with an increase of the inductive electron donating character of the 2-substituent on the side-chain that lowers the reactivity of the formed oxazolinium cation. Therefore, the k_p decreases in the order MeOx > EtOx > *n*PrOx, if one considers the same solvent-initiator system for accurate comparison[iv]. It is noteworthy that an increase of the side chain length for linear alkyl chains from ethyl to *n*-nonyl has a minimal influence on the k_p.[170] Amongst 2-oxazolines with a propyl side-chain the k_p decreases in the order *c*PrOx > *n*PrOx > *i*PrOx.[178] The CROP of 2-aryl-2-oxazolines is generally slower compared to 2-alkyl-2-oxazolines due to conjugation of the phenyl ring and the 2-oxazoline. Electron withdrawing *para*-substituents such as Cl or NO_2 slightly increase the k_p of 2-phenyl-2-oxazoline derivatives which can be explained by an increased reactivity of the cationic propagating species.[197] An exception to this general trend are *ortho*-fluoro substituted PhOx, whereby 2,6-difluoro-2-phenyl-2-oxazoline (2,6-DFOx) is the fastest 2-oxazoline monomer in the living CROP

[iv] will be assumed if kp´s are further discussed in this section of the chapter

reported to date.[202] This acceleration of the CROP of orthofluoro substituted PhOx is due to loss of the conjugation by out of plane twisting of the aryl substituent. For functional 2-oxazoline monomers, rate accelerations can also be observed such as *e.g.* in the work of Bouten *et al.,* where methyl ester containing 2-oxazolines (MestOx) were found to polymerize faster than MeOx, EtOx and PrOx monomers.[196] The acceleration was ascribed to the interactions of the oxazolinium chain-end with the neighboring MestOx units, thereby increasing the reactivity of the 5-position of the oxazolinium ring leading towards faster propagation. Furthermore, the MestOx units in the polymer stabilize the transition state for the additional of the next monomer.

Not only the monomer and polymerization temperature influence the k_p of the CROP of 2-oxazolines, but also the solvent nature determines the overall polymerization rate. Commonly, more polar solvents induce faster propagation through efficient solubilisation of the cationic chain-end species and in addition it ensures disruption of the ion paring. In contrast, the less polar solvents induce less chain transfer or side reactions (*vide supra* 1.3.3).[144, 153, 166,203] Up to date no systematic investigation on the effect of solvents on the CROP of 2-oxazolines has been reported. Consequently, this will be an important part of this thesis work (*vide* **chapter 2 and 3**), and serve as a basis for the synthesis of defined high molecular weight PAOx polymers.

Table 5. Overview of the propagation rate constants for the CROP of the most common 2-oxazolines (reformat from literature reference[148]). All polymerizations were carried out in acetonitrile at 140 °C with MeOTs as initiator (except for 2,6-DFOx which is carried out in nitromethane).

Monomer	k_p (in 10^{-3} L/mol.s)
MeOx	146 ± 3
EtOx	105 ± 1
***n*PrOx**	93 ± 2
***n*ButOx**	139 ± 2
***n*PentOx**	120 ± 3
***n*HeptOx**	127 ± 3
***n*NonOx**	111 ± 5
***i*PrOx**	47.8 ± 7
***c*PrOx**	257 ± 10
C2MestOx	171 ± 4
C3MestOx	193 ± 4
PhOx	32
2,6-DFOx	382.4

An important feature of the PAOx family is versatility, *i.e.* the fact that polymer properties can be controlled by variation of the side-chain and that functional groups can easily be incorporated in the polymer side-chains by employing functional monomers, as long as they are non-nucleophilic. Without going into too much detail, there exist a number of 2-oxazoline monomers containing heteroatoms such as, nitrogen, phosphor, oxygen and sulfur. The most important ones were already briefly described as methyl ester containing 2-oxazoline monomers, which can easily serve as a post-functionalization platform *via* direct amidation, referring to the work of Mees *et al.*[204] In general, functional monomers are not always straightforward to synthesize, disregarded from the fact that their polymerization conditions have to be carefully evaluated compared to the 2-alkyl-2-oxazoline monomers. To name one specific example, the monomers with a Boc protected amino group (C4NHBocOx) could only be polymerized using an oxazolinium salt as initiator while the use of methyl triflate resulted in the formation of undefined low molar mass products due to side reactions.[205]

As already mentioned, the choice of 2-oxazoline monomers for the CROP is only limited by the incompatibility of nucleophilic reactive groups, such as hydroxyls, amines, aldehydes, thiols and carboxylic acids, with the CROP. However, PAOx with these functional groups can be prepared by the CROP of ´protected´ monomers, including Boc protected amines and methyl esters, followed by post-polymerization deprotection (**Figure 21**).

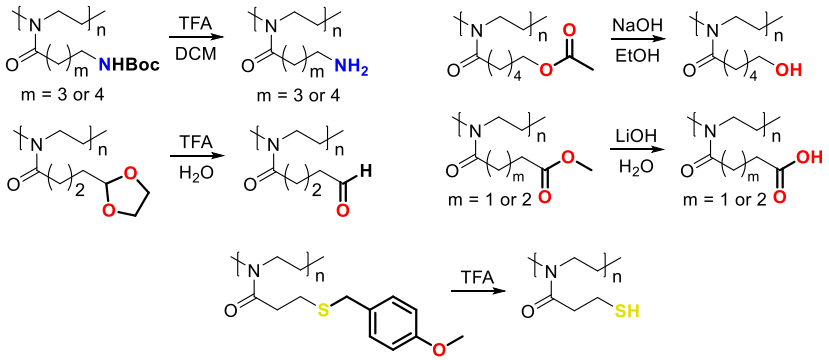


Figure 21. Synthesis routes for PAOx with amino, carboxy, hydroxyl, aldehyde and thiol groups in the side-chains by post-polymerization deprotection.

Finally, 2-oxazoline monomers with perfluoroalkyl substituents have been reported, which can be used for the preparation of extremely hydrophobic PAOx. 2-Perfluoroalkyl-2-oxazolines were found to be much less reactive than 2-alkyl-2-oxazolines and therefore difficult to polymerize.[206]

Even though a large number of (functional) 2-oxazoline monomers and PAOx homopolymers have been reported, only few of them are of interest for this PhD project, in terms of ease of synthesis, scalability, properties, *etc.* (*vide infra*). In this aspect, for the SD formulations of poorly water-soluble APIs PMeOx, PEtOx and P*n*PrOx with a MW of 50,000 g/mol are of interest, since a water-soluble matrix, that can undergo some hydrophobic interactions with the hydrophobic API, is preferably used in order to increase their water solubilization rate. In contrast, higher MW and/or more hydrophobic polymers, such as 140,000 g/mol PEtOx or 50,000 to 100,000 g/mol P*n*PrOx matrices are desired for the formulation of highly water-soluble APIs to slow down their solubilization in SRF.

1.3.5 Thermal properties of poly(2-oxazoline) homopolymers

The alkyl-PAOx polymers are stable up to temperatures of approximately 300 °C and more[171,193] enabling applications and processing over a broad temperature range. Alkyl-PAOx with varying length of the side-chains have been widely studied in terms of their structure-property relationships. **Figure 22** shows an overview of the T_g and melting temperatures (T_m) obtained from differential scanning calorimetry (DSC) of alkyl-PAOx with 1-17 carbon atoms in the side-chain.

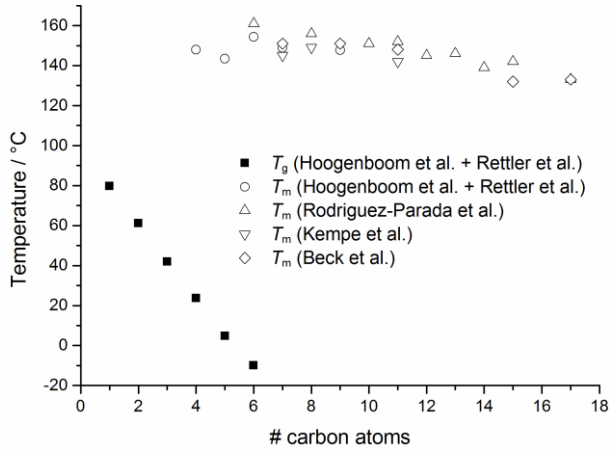


Figure 22. Glass transition and melting temperatures of poly(2-n-alkyl-2-oxazoline)s with varying side-chain length obtained from DSC. Data are taken from refs. [128, 170, 172, 182,187]

PAOx polymers with 1-5 carbon atoms in the side-chain (PMeOx to P*n*PentOx) show a T_g that decreases linearly with increasing side-chain length which can be explained by the increasing flexibility of the side-chains. For PMeOx, PEtOx and P*n*PrOx no melting peaks were observed by differential scanning calorimetry, while polymers with 4 or more carbon atoms in

the side-chain were found to be semi-crystalline with a T_m around 150 °C independent of the side-chain length indicating side-chain crystallization. In contrast, Litt and co-workers[113] reported melting temperatures also for PMeOx and P*n*PrOx by differential temperature analysis (DTA) based on a difference in birefringence. This discrepancy might originate from different processing of the polymers, potentially resulting in alignment of the main-chains and crystallization if enough time is given.[207,208] Remarkably, the fatty-acid based PSoyOx revealed a much lower T_m (88.4 °C) than PAOx with long linear saturated alkyl side-chains due to the *cis*-double bonds in the side-chains that disturb the crystallization and results in the lower T_m.[209] Fully amorphous PAOx with a low T_g can be prepared using monomers with branched alkyl side-chains as demonstrated for poly(2-(1-ethylpentyl)-2-oxazoline) (EPOx), poly(2-(3-ethylpentyl)-2-oxazoline) (P3EPOx) and poly(2-(3-ethylheptyl)-2-oxazoline) (PEHOx) (**Figure 23**), the latter being the amorphous poly(2-oxazoline) with the lowest reported T_g to date, being -6 °C, which may be suitable to prepare rubbery-like block copolymers, analogous to styrene-butadiene-styrene (SBS), materials with a soft middle block.[210]

PAOx with cycloalkyl side-chains exhibit interesting thermal properties. While PcPrOx is amorphous with a higher T_g (79 °C) than its linear analogue P*n*PrOx;[192] PcButOx, PcPentOx and PcHexOx are semi-crystalline high performance polymers with an extraordinary high T_m of 243, 251 and 306 °C, respectively.[193] Noteworthy, the degradation temperature of PcButOx and PcPentOx is approximately 100 °C higher than their T_m, making them promising candidates for application as heat-resisting materials with a large processing window. In addition, also high melting points have been reported for adamantyl-containing PAOx.[211] Furthermore, poly(2-*n*-perfluoroalkylethyl)-2-oxazoline)s represent very hydrophobic PAOx. Interestingly, their melting temperatures are much higher compared to their hydrocarbon analogues and unlike the alkyl-PAOx, the melting points of the perfluoro-PAox increase as the side-chain length increases, as can be found in work from Rodriguez-Parada *et al*.[182]

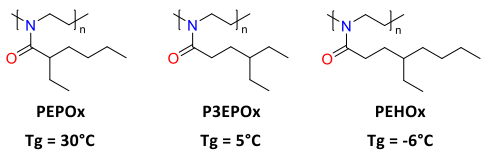


Figure 23. Structures of low T_g PAOx polymers with alkyl branched side-chains; *i.e.* poly(2-(1-ethylpentyl)-2-oxazoline) (EPOx), poly(2-(3-ethylpentyl)-2-oxazoline) (P3EPOx) and poly(2-(3-ethylheptyl)-2-oxazoline) (PEHOx).

Next, by looking at phenyl containing PAOx polymers, such as PPhOx and fluoro-substituted phenyl PAOx, a higher T_g (between 103 °C and 135 °C, respectively), compared to PMeOx and PEtOx, is found as a consequence of the rigid aromatic phenyl ring present in all the

polymers. [113, 128, 171,212] Finally, if one has a look towards functional PAOx polymers, which have been synthesized and investigated with regard to thermal properties, it is found that all reported functional polymers are amorphous, thus possess only a T_g. For example, the methyl ester containing PAOx, C2MestOx and C3MestOx, have a T_g of 39 °C and -1 °C, respectively; as been published by Bouten *et al.*[194] A more complete table of all T_g´s reported for functional PAOx, can be found in the review by Glassner *et al.*[158]

Mechanical properties of poly(2-oxazoline) homopolymers

There are only very few reports on the mechanical properties of PAOx homopolymers as the majority of polymers have a rather low molar mass leading to very poor (brittle) mechanical properties, thereby obstructing mechanical testing. The mechanical properties of low molar mass alkyl-PAOx (DP = 60) have been reported based on nano-indentation of spin-coated polymer films. These measurements revealed that the mechanical properties are closely related to the trends seen in the thermal properties of alkyl-PAOx (*vide supra,* **Figure 22**). Upon increasing the length of the alkyl side-chain from methyl to butyl, the elastic modulus at room temperature decreases almost linearly[172,207] (**Figure 24**) related to the decrease in T_g.

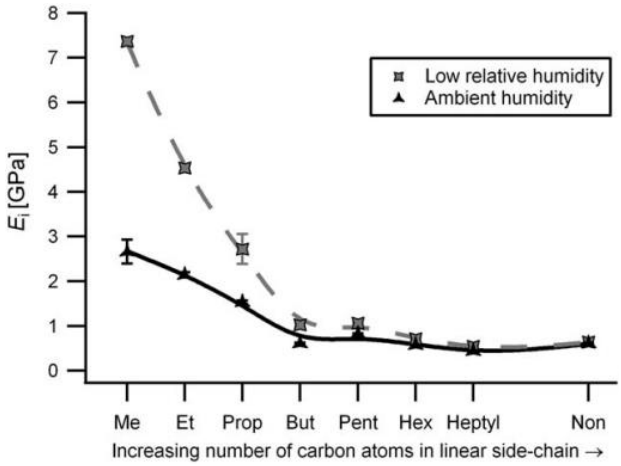


Figure 24. Indentation moduli of poly(2-*n*-alkyl-2-oxazoline)s with DP ~ 60 at low and ambient humidity. The lines are added to guide the eye only. Reproduced from Ref.[207]

The polymers with more than four carbon atoms in the side-chain are semi-crystalline and have a T_g below ambient temperature. Therefore the measurements at room temperature were performed above their respective T_g resulting in moduli of *ca.* 0.8 GPa, which is common for polymers tested between their glass transition and melting temperatures.[213] It should be noted

that the mechanical properties of PMeOx, PEtOx and P*n*-PrOx strongly depend on the humidity due to the hygroscopic nature of these polymers. As can be seen from **Figure 24**, the plasticizing effect of incorporated water significantly reduces the indentation moduli.[207] It is expected that more in depth mechanical properties of PAOx will become available in the near future based on the fact that defined high molar mass PAOx have become synthetically accessible.

1.3.6 Solution properties of poly(2-oxazoline) homopolymers

The variation of the side-chain structure of PAOx enables the tuning of the properties from very hydrophilic (PMeOx) to very hydrophobic (long (branched or linear) alkyl or perfluoro alkyl side-chains) polymers. Consequently, the solution behavior of PAOx strongly depends on the side-chain structure. **Table 6**, gives an overview of the solubility of various alkyl-PAOx in water and common organic solvents, such as methanol, chloroform, acetonitrile, *etc*. Obviously, to prepare defined PAOx polymers *via* the living CROP it is necessary that both monomer and polymer structure are soluble at all times during the polymerization.[166] Some reports, *e.g.* on the polymerization of poly(2-*iso*-butyl-2-oxazoline), show uncontrolled polymerization as a consequence of phase separation occurring during the CROP.[188]

In addition to the solvents listed in **Table 6**, the less common solvent sulfolane was found to be a good solvent for preparing high molar mass PMeOx, *vide* **chapter 2**.[214] In contradiction to the good solubility of the series of alkyl-PAOx, the semi-crystalline polymers, *i.e.* PcBuOx, PcPentOx and PcHexOx, show poor solubility in many common organic solvents, but are found to be soluble in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and formic acid at ambient temperature that disrupt interchain interactions.[193] The behavior of PAOx in aqueous solutions has been widely studied, because of their potential in biomedical applications. Mainly, their behavior is influenced by the hydration of the polymer and the accompanied loss in entropy. With increasing temperature, the entropy loss increases and at the phase transition temperature it becomes more favorable to release the water molecules into the bulk water resulting in phase separation of the polymers chains (**Figure 25**).

Therefore, many PAOx exhibit a lower critical solution temperature (LCST). Two comprehensive reviews about thermoresponsive PAOx by Weber *et al.*[215] and Hoogenboom and Schlaad[216] give an excellent overview on these polymers and their solution behavior.

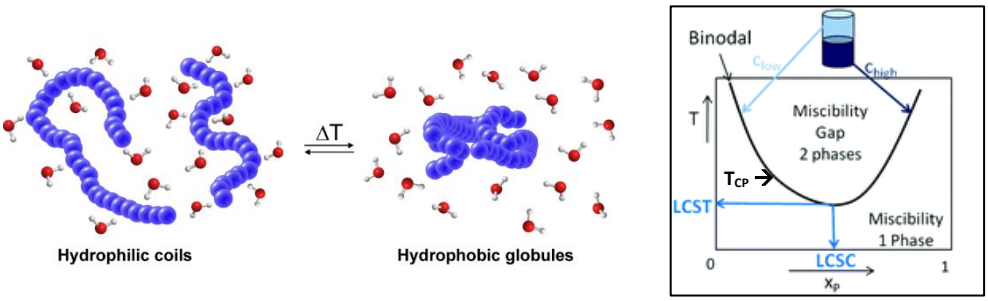


Figure 25. Schematic representation of a polymer phase transition in aqueous solution, as in lower critical solution behaviour (**Left**); scheme of a LCST phase transition diagram presented as the temperature as a function of polymer concentration in a particular solvent with LCSC the lower critical concentration at the LCST transition and T_{CP}, as the cloud point temperature at another concentration anywhere else on the graph apart from the lowest point, which is the LCST. Adapted from ref. [217,218]

Table 6. Solubility of PAOx with hydrocarbon side-chains in water and various organic solvents.

	Water	Methanol	Chloroform	Acetonitrile	*N,N*-dimethyl formamide	Chlorobenzene	*N,N*-dimethyl acetamide
PMeOx	+	+	-	+/-[a]		-	+
PEtOx	LCST	+	+	+	+	+	+
PnPrOx	LCST	+	+	+		+	+
PnNonOx	-		+	-	-		+
PPhOx	-		+	+	+		+
PcBuOx	-	-		+/-[b]		+/-[b]	+/-[b]
PcPentOx	-	-		-		-	-

+ = soluble, - = insoluble, +/- = partially soluble, LCST = lower critical solution temperature, [a]poor solubility for molar mass >~ 20kDa, [b]soluble after heating, precipitated on cooling, [c]soluble after heating

PMeOx has been claimed to be even more hydrophilic than PEG based on HPLC analysis[79] as well as based on the poor solubility of higher molar mass PMeOx in organic solvents like acetonitrile in which higher molar mass PEG is well soluble.[214] As a result, PMeOx is fully water soluble from 0-100 °C while PAOx with longer alkyl side chains are either water insoluble or are only soluble in water at a certain concentration, *i.e.* exhibit LCST behavior. An overview of the cloud point temperatures (T_{CP}, the temperature where the transmittance rapidly decreases due to phase separation of the polymer) of PAOx homopolymers with increasing hydrophobicity is given in **Figure 26**. It should be noted that the T_{CP} depends on the molar mass, the concentration, the polymer end-groups and the conditions of the measurement[218] and therefore comparisons

of T_{CP}'s reported in the literature should be handled with care, as Zhang *et al*. reported very recently.[218]

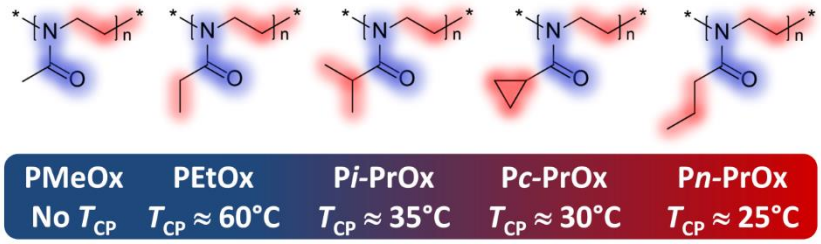


Figure 26. PAOx with hydrocarbon side-chains with increasing hydrophobicity (from left to right) and consequently decreasing cloud point temperature.

The LCST behavior of high molar mass PEtOx was first reported in 1988 by Lin *et al.* with T_{CP}'s from 61-69 °C dependent on the concentration.[219] Lowering the molar mass of PEtOx homopolymers results in a significant increase of the T_{CP} in aqueous solution.[177,220]

Amongst thermo-responsive polymers, those with a LCST close to body temperature are of special interest for applications in drug delivery or bioengineering.[41,221] Interestingly, PAOx with propyl side-chains namely P*n*PrOx, P*c*PrOx and P*i*PrOx have a T_{cp} in this range. It was first reported in 1992 that P*i*PrOx exhibits LCST behavior with the T_{CP} decreasing from 39 to 35 °C with increasing polymer concentration (0.1 – 1 wt%).[184] More detailed investigations of P*i*PrOx with varying molar mass revealed that the T_{CP} decreases with increasing molar mass and that the concentration dependence becomes less pronounced with increasing molar mass.[160,161,186] All of these studies found the phase transition to be reversible with a small heating-cooling hysteresis. However, it was later discovered that the transition becomes irreversible after keeping the dispersion longer above the cloud point due to isothermal crystallization of the (partially) dehydrated polymer chains.[222,223]

It was recently shown that cyclic P*i*PrOx exhibits a much higher T_{CP} compared to linear P*i*PrOx with the same molar mass.[224] As much as 15 years after the LCST behavior of P*i*PrOx was discovered, it was reported that P*n*PrOx also exhibits LCST behavior in aqueous solutions.[95] The T_{CP} was found to be more than 10 °C lower compared to P*i*PrOx with a similar molar mass which is in accordance with the higher hydrophobicity. As previously observed for P*i*PrOx, the T_{CP} of P*n*PrOx also decreases with increasing molar mass.[177] The cyclic side-chain structure of P*c*PrOx results in an intermediate hydrophobicity compared to P*i*PrOx and P*n*PrOx and consequently its T_{CP} in aqueous solution was found to be in between the T_{CP}'s observed for P*i*PrOx and P*n*PrOx.[191,192]

In contrast to P*i*PrOx the phase transitions of the amorphous P*n*PrOx and PcPrOx are fully reversible, even when kept above T_{CP} for a prolonged time. PAOx with methyl ester side-chains were first reported 1968,[150] but it was only recently reported that these polymers exhibit thermo-responsive LCST behavior in aqueous solution.[224] Their T_{CP}'s strongly depend on the length of the alkyl spacer. PC2MestOx shows a very similar solution behavior as PEtOx with a T_{CP} around 100 °C for a polymer with DP 100 while PC3MestOx exhibits a T_{CP} around 25 °C similar to P*n*PrOx. In contrast to the LCST behavior, there are no poly(2-oxazoline)s reported that show an upper critical solution temperature (UCST) in water. However, UCST behavior was observed for PAOx with phenyl, benzyl or alkyl side-chains longer than propyl in ethanol-water mixtures,[224] which results from the change in polarity of these non-ideal solvent mixtures upon heating.[225] **Figure 27** shows an overview of the solubility of various PAOx in ethanol-water mixtures.

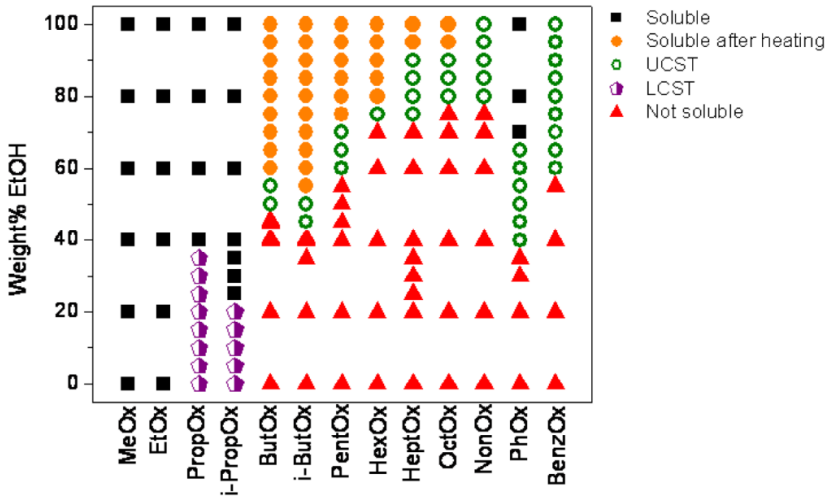


Figure 27. Solubility overview for poly(2-oxazoline)s with hydrocarbon side-chains in water-ethanol mixtures (5 mg/mL). Reproduced from ref. [224]

1.3.7 Application of poly(2-oxazoline)s in the biomedical field

Almost 25 years after the ´discovery´ of the CROP of 2-oxazolines and resulting PAOx polymer structures, studies by D´ejardin *et al.* and Goddard *et al.*[226,227] in 1989 showed a first indication of protein resistance, later called ´stealth behavior[v]´, of PAOx (co)polymers based on investigation of the biodistribution of (radio-labeled) PMeOx polymers in mice. Furthermore, Zalipsky *et al.* and Woodle *et al.* reported on enhanced blood circulation times of PMEOx and

[v] Stealth literally means ´making invisible´; related to drug delivery a material that possesses stealth behavior can be regarded as non-recognizable by the immune system.

PEtOx polymers in the 1990s, similar to the ´gold standard´ in the field PEG/PEO polymers.[228,229] Additional studies on the biodistribution of PAOx polymers followed approximately 15 years later with the work of Gaertner *et al.* and very recently the renal clearance limit of PEtOx was reported to be around 40 kDa.[230,231] Since the years 2000, the number of literature papers and reviews on the use of PAOx polymers in biomedical applications increased tremendously, which can be elucidated from the graph in **Figure 28**. From 2007 up to now, over 300 publications on PAOx in a biomedical context have been published, with a number of interesting reviews by Hoogenboom *et al.*, Verbraeken *et al.*, Schlaad *et al.*, Adams *et al.* and Sedlacek *et al.* describing the high potential of PAOx and their use in all types of biomedical applications.[109, 118,119, 134,164,165] A wide range of applications is described with a focus on PAOx-protein and drug conjugates,[122,232–236] PAOx-based micelles for drug encapsulation (non-covalently) and triggered drug release,[200,237–239] as well as hydrogels (**Figure 29**).[238,240] Another important application of PAOx can be found in gene delivery for which it serves as a precursor for the synthesis of linear poly(ethylene imine) (PEI) or PEtOx-EI copolymers. Different studies showed or described the improved effect of gene therapy by the use of linear PEI over branched PEI.[118, 153,241–244] In addition, an important study, by De Geest *et al.*, related to the acid hydrolysis of PEtOx revealed their chemical resistance over more than 40 hours, under conditions of the gastrointestinal tract (GI tract), which is quite essential towards the use of PAOx in the human body.[245]

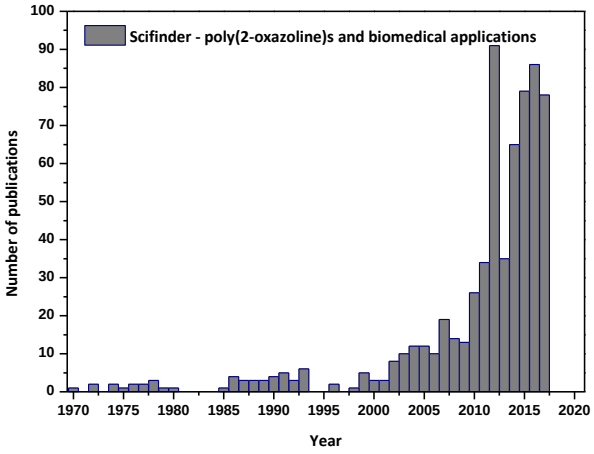


Figure 28. Number of publications, over the last 50 years, including poly(2-oxazoline)s and biomedical applications, according to entry in Scifinder®.

It will be clear that the use of a large variation of PAOx (co)polymer architectures and designs have been reported related to drug or protein delivery. However, only very few examples can be found for the application of PAOx as matrix excipient for solid dispersions or sustained release formulations. In 2012, Claeys *et al.* published work based on HME/IM of

Aquazol® (*i.e.* poorly defined PEtOx, Đ approximately 3-4) of poorly and good water-soluble drugs[110] and in that same year Bender *et al.*[246] filed a patent on the protection capabilities of Aquazol® for cannabinoids. Also, Policianova *et al*. published two papers on acetylsalisylic acid solid dispersions, including comparison between state-of-the-art excipients, such as PVP, PEG, and PEtOx polymers.[247,248] Despite these promising results, the lack of further use of PAOx polymers as matrix excipient may be related to the inability to synthesize defined PAOx (co)polymers with a molecular weight higher than 20,000 g/mol; which will be necessary for further widespread application. Higher MW PAOx polymers are desired for better processability and mechanical properties of the polymers resulting from chain entanglements.

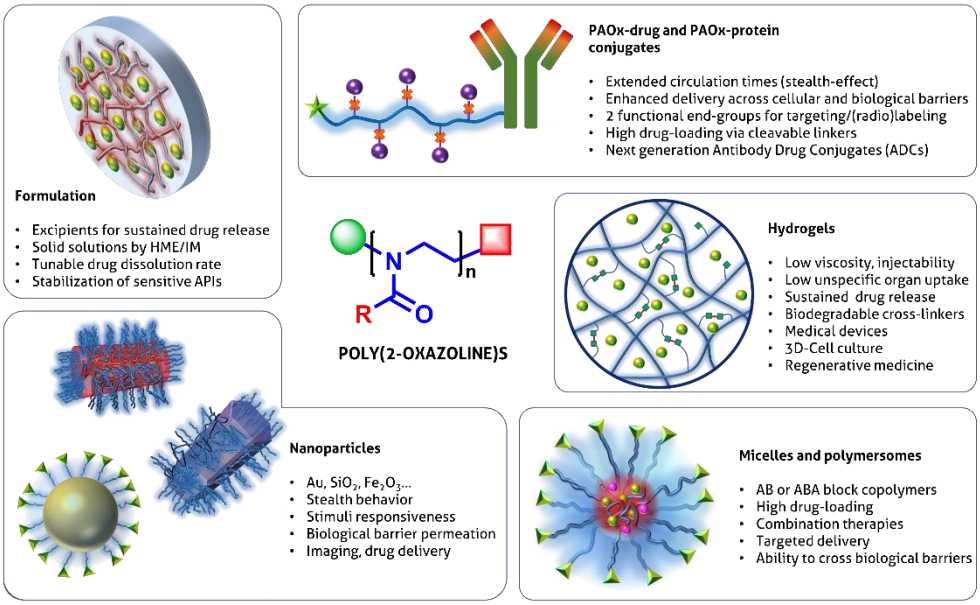


Figure 29. Overview of the different architectures and designs based on poly(2-oxazoline)s for the application in the biomedical field. [249]

1.4 CONCLUSIONS FROM LITERATURE AND OUTLINE THESIS

Based on the literature review it can be concluded that the use of biocompatible polymers in oral drug formulations is a growing research field with enormous potential. Nonetheless, the state-of-the-art polymers that are used do not provide a tunable platform for the formulation of a wide variety of APIs. Introducing the PAOx polymer family in this field, as a tunable polymer platform for oral drug formulation of APIs, may further stimulate the use of polymers as matrix excipient. The summary of advantages for PAOx polymers include their high chemical and physical stability, their tunability and easy processing. It is important to mention that the wide variety of potential structures can also be seen as a disadvantage as the possibilities are indefinite. The ideal drug carrier or polymer carrier should succeed in release of the drug in the appropriate time frame, *vide* sustained or immediate release, provides long-term shelf-life stability, *i.e.* after at least one year it should provide the same release profile and possess the same properties as measured after processing. Furthermore, a polymer carrier should be able to be used for formulation of a wide range of APIs.

In this thesis a combination of polymer synthesis and pharmaceutical research is performed to explore PAOx as matrix excipient for oral drug formulations, dividing the thesis work in a polymer synthesis part and a pharmaceutical applications part (overview in **Figure 30**).

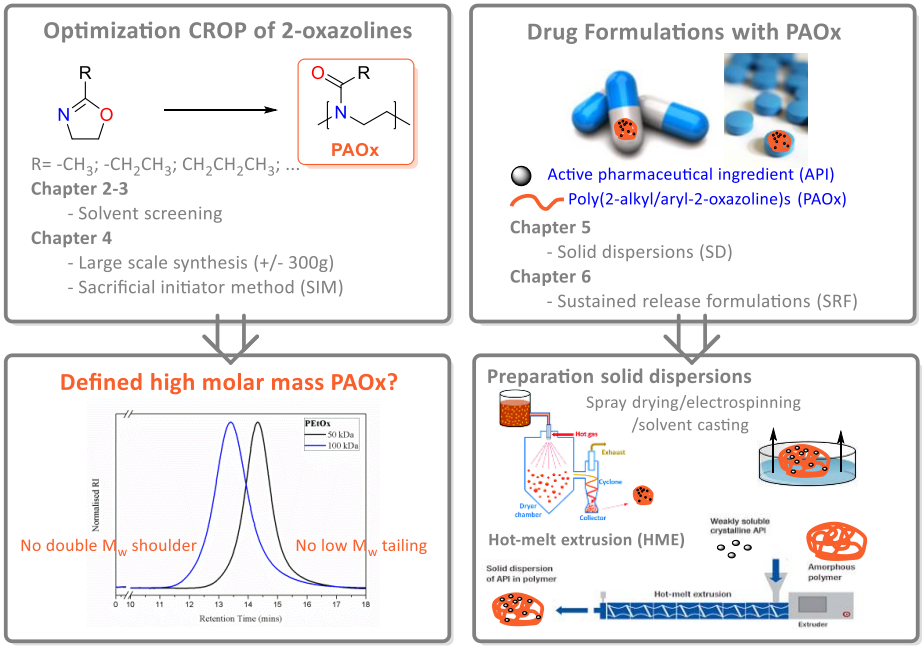


Figure 30. Schematic outline thesis existing of a synthesis part (chapter 2-4) and a drug formulation part (chapter 5-6).

In the first part, fundamental research has been done on the optimization and up-scaling of the CROP of 2-oxazolines. In **chapter 2**, the route towards defined poly(2-methyl-2-oxazoline) is described, including polymerizations carried out in extremely polar solvents such as sulfolane. Further, in **chapter 3**, the optimization of the CROP for the preparation of the most used hydrophilic and biocompatible polymer in the PAOx familiy, *i.e.* poly(2-ethyl-2-oxazoline), is investigated at different temperatures with regard to different solvents ranging from apolar to polar. A first step towards the green synthesis, in terms of biocompatibility, is set by using ethyl acetate as non-harmful solvent for the polymerization of 2-ethyl-2-oxazoline.

In **chapter 4**, upscaling of the synthesis of defined high molar mass PAOx is highlighted. The importance of reproducible synthesis for the defined high MW PAOx is stressed, together with some pitfalls and difficulties in obtaining them.

In the second part of the PhD thesis, the focus is put on the pharmaceutical applications of these defined high MW PAOx synthesized in the previous chapters. In **chapter 5**, the use of PAOx polymers in solid dispersions is described. Mainly, the influence of different PAOx polymers have been evaluated for the formulation of poorly water-soluble drugs and the corresponding increased release rate and solubility of the drug. Finally, in **chapter 6**, more hydrophobic PAOx polymers are tested in drug formulations of highly water-soluble APIs, in the so-called sustained release formulations. The obtained results show a remarkable difference in the use of PEtOx compared to poly(2-*n*-propyl-2-oxazoline)s and poly(2-*sec*-butyl-2-oxazoline)s, for controlled/extended release of the used model APIs.

In **chapter 7**, conclusions and future perspectives will be given on the use of defined (high molecular weight) PAOx polymers as matrix excipient for solid dispersions and sustained release formulations, *i.e.* oral drug formulations.

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Chapter 2

CHAPTER 2

2 A JOURNEY TOWARDS DEFINED POLY(2-METHYL-2-OXAZOLINE)

ABSTRACT The synthesis of poly(2-methyl-2-oxazoline)s (PMeOx) and poly(2-oxazoline)s (PAOx) in general, dates back from the late 1960s.[1,2] Thereby it was already clear that interfering impurities would play a crucial role in the cationic ring-opening polymerization (CROP) of 2-oxazolines, as clearly reported by Litt and coworkers.[3] In this chapter first the challenges, either found in literature or experimentally determined in my PhD, associated to the CROP of MeOx will be discussed. Herein, the search for alternative solvents for the cationic ring-opening polymerization (CROP) of 2-methyl-2-oxazoline (MeOx) is driven by the poor solubility of higher molar mass PMeOx in polymerization solvents such as acetonitrile (CH_3CN) and chlorobenzene as well as in MeOx itself. Next, the polymerization of MeOx in sulfolane, as most suitable but rather exotic solvent, is described. Since this solvent is quite rare within polymerizations of any kind, an exclusive part is dedicated towards its use. Unexpectedly, an increased propagation rate constant (k_p) was found for the CROP of MeOx in sulfolane. Further extended kinetic studies at different temperatures (60 °C- 180 °C), revealed that the acceleration is due to an increase in frequency factor while the activation energy (E_a) of the reaction is unaffected compared to the CROP in CH_3CN. In order to explore the versatility of sulfolane as polymerization solvent for the CROP of 2-oxazolines in general, also the polymerization kinetics of other 2-oxazoline monomers, such as 2-ethyl-2-oxazoline (EtOx) and 2-phenyl-2-oxazoline (PhOx), have been studied in sulfolane resulting in a general acceleration of the CROP of these monomers as well as a PMeOx-b-PhOx block copolymer. Finally, a straightforward synthesis for a defined 50,000 g/mol PMeOx polymer in sulfolane is elaborated at the end of this chapter to emphasize the importance of sulfolane as enabling solvent for preparing poly(2-methyl-2-oxazoline)s (PMeOx) (co)polymers. PMeOx is especially relevant for biomedical applications as most hydrophilic polymer in the poly(2-alkyl-2-oxazoline) family.

Contributors to this work

Bart Verbraeken and Bryn D Monnery were involved in conceptual discussions.

Contribution of the candidate

Performed all the experiments and wrote first author paper:

Vergaelen, M *et al.*, Sulfolane as common rate accelerating solvent for the cationic ring-opening polymerization of 2-oxazolines, ACS Macroletters, **2015**, 4, 85-828.

Chapter 2

2.1 TROUBLESHOOTING THE CROP OF 2-METHYL-2-OXAZOLINE: INTRODUCTION

During the past decades, researchers have been searching for alternative biocompatible polymers that 'outperform' the gold standard in the field, poly(ethylene glycol).[4–6] In this respect, poly(2-alkyl/aryl-2-oxazoline)s (PAOx), more specifically poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx), have been widely investigated for their use in biomedical applications.[4,7–9] PAOx are prepared by living cationic ring-opening polymerization (CROP) of 2-oxazolines and their polymerization mechanism has been studied widely (*vide* **chapter 1**).[10] One of the main advantages of the CROP of 2-oxazolines is that its living nature provides good control over the PAOx molar mass distribution, *i.e.* narrow dispersity (Đ), and very high end-group fidelity.[1,11] Additionally a broad range of structures and polymer properties are easily accessible by variation of the monomer structures.[12–15] The CROP of 2-oxazolines consists of three steps, namely the initiation, propagation and termination as discussed in **chapter 1** (**Figure 1**).[16–23] The CROP of 2-oxazolines is often proposed to occur in an ideal living manner assuming that no chain transfer and termination reactions occur during the polymerization.[12,24,25]

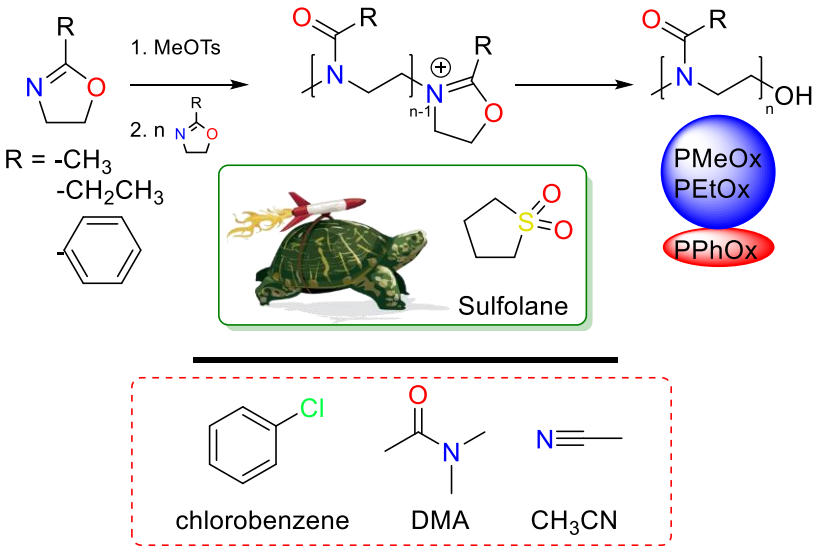

Figure 1. Table of content of the respective article published on the use of sulfolane as a 'universal' solvent for the cationic ring-opening polymerization of poly(2-alkyl/aryl-2-oxazoline)s.

The propagation rate constant (k_p) of the CROP of 2-oxazolines determines the rate of the overall polymerization and is influenced by different parameters such as the type of monomer, initiator and solvent.[26–31] In earlier research, by, amongst others, the groups of Litt and Nuyken, a variety of parameters have already been discussed.[1, 25,32] Litt explored the effect and possible interference of solvents with different functional groups on the CROP of 2-oxazolines, in order to

explain the mechanism of polymerization. In this early research 2,4-dimethyl sulfolane was already mentioned as a non-interfering solvent, which was proven by a normal infrared spectrum of the polymer that was obtained for the CROP of 2-(n-pentyl)-2-oxazoline in 2,4-dimethyl sulfolane.[33,34]

The CROP of 2-oxazolines is most commonly performed in CH_3CN,[33,35] using MeOTs as initiator. However, CH_3CN seems to be less suitable for the polymerization of MeOx due to the poor solubility of PMeOx in CH_3CN, resulting in reduced control over the CROP of MeOx due to phase separation when aiming for higher degrees of polymerization (DP's). The low solubility of PMeOx in CH_3CN can be explained by a significant change in hydrophilicity upon polymerization due to the isomerization of the cyclic imino ether structure (MeOx) to a tertiary amide structure (PMeOx). The same reasoning can be applied to explain the poor solubility of PMeOx in other common polymerization solvents, including chlorobenzene and even its own monomer, obstructing bulk polymerization.

According to Tilstam *et al.*, sulfolane is a quite eccentric solvent compared to other dipolar aprotic solvents such as *N,N*-dimethyl acetamide, dimethyl sulfoxide, *etc.* It is mostly used in oil refineries, for the recovery of high- purity aromatic products and the purification of gas streams. If one looks at its characteristics it is an extremely high boiling solvent, has the ability to dissolve strong cations and polarizable intermediates because of its strong solvency power and it dissolves in almost any organic/inorganic medium, except for *tert*-butyl methyl ether.[35] If one looks at the use of sulfolane as polymerization solvent only one recent publication by Mendes *et al.*[36] describes its use in copper mediated ATRP as universal solvent.

In order to tackle the optimization of the CROP of MeOx to obtain defined high MW PMeOx a solvent screening will be carried out to check alternative solvents compared to the CROP of MeOx in acetonitrile (**section 2.3.1**). In **section 2.3.2**, sulfolane is explored as a valuable alternative for the CROP of MeOx. Therefore, a complete set of kinetic experiments at different temperatures is set up, to investigate the CROP of MeOx in sulfolane. Furthermore, the CROP of EtOx and PhOx in sulfolane are studied, to question the use of sulfolane as common solvent for the CROP of 2-oxazolines. In order to verify the livingness of the CROP of 2-oxazolines in sulfolane the synthesis of a poly(MeOx-block-PhOx) copolymer is reported in sulfolane (**section 2.3.3**). Finally, in **section 2.3.4**, the synthesis of a defined 50,000 g/mol PMeOx polymer is described in sulfolane.

2.2 MATERIALS AND METHODS

2.2.1 Materials and equipment

Materials

Methyl *p*-toluenesulfonate (MeOTs), 2-phenyl-2-oxazoline (PhOx) and solvents (sulfolane, *N,N*-dimethylacetamide (DMA), acetonitrile (CH_3CN), chlorobenzene) (HPLC quality) were purchased from Sigma-Aldrich. 2-Methyl-2-oxazoline (MeOx), barium oxide and potassium hydroxide were purchased from Acros Organics. 2-Ethyl-2-oxazoline (EtOx) was kindly donated by Polymer Chemistry Innovation. CH_3CN was purified over aluminum oxide by means of a solvent purification system from J.C. Meyer.

Purification methods

All 2-oxazolines were distilled over barium oxide under inert argon atmosphere (MeOx and EtOx) or under reduced pressure (PhOx) before use in the polymerizations. MeOTs was distilled over CaH_2 under reduced pressure. Purification of sulfolane was performed according to the procedure described in the book 'Purification of Laboratory Chemicals', 6th edition.[37] Initially, small portions of solid $KMnO_4$ were added to the sulfolane (at 50 °C) until the color remains up to one hour. This is mainly to deal with 3-sulfolene and 2-sulfolene impurities. Thereafter, a dropwise addition of MeOH is performed to destroy the excess of $KMnO_4$ followed by filtering the solution. Note: to clean the filter afterwards (MnO_2 is quite harsh to remove) concentrated sulfuric acid was used. Trace amounts of MeOH were removed on the rotavap for a few minutes. Then CaH_2 was added as a drying agent and the mixture was stirred overnight. Afterwards a distillation was carried out under high vacuum (hot plate > 90 °C and 10^{-2} mbar), without any condenser (sulfolane becomes a solid at ca. 30 °C). The whole distillation set-up was covered in aluminum foil to increase the heat transfer and only the receiving flask was cooled with ice.

Equipment

Conversions of the CROP of the selected 2-oxazoline monomers were monitored by gas chromatography (GC) analysis. GC was performed on an Agilent 7890A system equipped with a VWR Carrier-160 hydrogen generator and an Agilent HP-5 column of 30 m length and 0.320 mm diameter. An FID detector was used and the inlet was set to 240 °C with a split injection of ratio 25:1. Hydrogen was used as carrier gas at a flow rate of 2 mL/min. The oven temperature was increased with 20 °C/min from 50 °C to 120 °C, followed by a ramp of 50 °C/min. to 240 °C.

All stock solutions and samples were prepared in a VIGOR Sci-Lab SG 1200/750 Glovebox System with an atmospheric water content ≤ 0.1 ppm. For the polymerizations, a Biotage Initiator EXP Microwave System with Robot Sixty was used. During the polymerizations the microwave synthesizer operated at a constant set temperature which is monitored by the IR-sensor.

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50 °C equipped with two PLgel 5 µm mixed-D columns in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent is DMA containing 50 mM of lithium chloride at an optimized flow rate of 0.593 ml/min. The spectra were analyzed using the Agilent ChemStation software with the GPC add on. Molar mass and dispersity (Đ) values were calculated against polymethylmethacrylate standards from PSS.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance 300 MHz spectrometer, at room temperature. The chemical shifts are given relative to trimethylsilane (TMS).

2.2.2 *Experimental methods*

Kinetic studies of the CROP

To perform the kinetic studies, 45 mL stock solutions have been made and divided over different 2.5 mL microwave vials, each filled with 700 µL to 800 µL of this stock solution. For each kinetic run at a certain temperature, 7 microwave vials have been filled and heated for a certain time. The stock solutions (**Table 1**) consist in general of a 3 M monomer (MeOx, EtOx or PhOx) concentration, 30 mM initiator (MeOTs) concentration and sulfolane as the polymerization solvent (except the polymerizations in CH_3CN and DMA). All calculations have been done in order to synthesize polymers with a M:I ratio, *i.e.* a target degree of polymerization (DP), of 100.

Table 1: Calculations for the kinetic study for the homopolymerization of MeOx, EtOx and PhOx.

Monomer	[M]/[I]	[M]	V (Stock, total)	[I]	V (I)	V (M)	V (S)
Name	ratio	mol/L	mL	mol/L	µL	mL	mL
MeOx	100	3	45	0.03	210	11.5	33.3
EtOx	100	3	45	0.03	210	13.7	31.1
PhOx	100	3	45	0.03	210	17.7	27.1

*M= Monomer; I= Initiator; S = Solvent [ ] = concentration; V( ) = Volume

After the reaction, GC samples were made by sampling 100 µL of the reaction medium into a sample vial and adding 900 µL of chloroform, as non-interfering solvent. For the SEC analysis, the samples were terminated with 500 µL of a methanolic KOH solution (1 M), followed by 100 µL sampling of the reaction medium and addition of 900 µL of DMA. All samples were carefully filtered over 0.13 µm filters (FE1322) before SEC analysis. In **table 2** the Arrhenius parameters resulting from the kinetic studies are listed. The one second sample is regarded as time zero were the reaction temperature reached 140 °C.

Table 2: Overview of the Arrhenius parameters as a function of monomer and solvent; activation energy and frequency factor.

Monomer	Solvent	Activation energy*	Frequency factor*
		kJ/mol	**A (10^8 $Lmol^{-1}s^{-1}$)**
MeOx	sulfolane	81.1 ± 2.7	64.0 ± 2.5
MeOx	CH_3CN	75.4 ± 0.5	5.1 ± 1.2
EtOx	sulfolane	74.3 ± 1.6	4.8 ± 0.1
EtOx	CH_3CN	73.4 ± 0.5	2.0 ± 0.9
PhOx	sulfolane	85.2 ± 4.7	31.5 ± 2.0
PhOx	CH_3CN	84.4 ± 0.5	14.9 ± 2.8

*The activation energy and frequency factor are based on data sets at five different temperatures. The data for acetonitrile are gathered froma literature reference by Wiesbrock *et al.*[12]

Synthesis of poly(2-methyl-2-oxazoline)$_{50}$-block-(2-phenyl-2-oxazoline)$_{50}$

The synthesis of this block copolymer (DP 100) consists of a two-step one-pot reaction. In a 10 mL microwave vial, a stock solution of MeOx (1.67 mL), MeOTs (0.061 mL) and sulfolane (4.78 mL) was prepared and reacted for 5 min at 140 °C. The reaction time was calculated according to the kinetic data of the homopolymerization of MeOx. After polymerization of the first block, a SEC sample was made of the prepared PMeOx block with DP 50 followed by sequential addition of the second monomer, PhOx (1.18 mL). This polymerization mixture was heated for 14 min at 160 °C under diluted conditions (1 M; sulfolane 4.18 mL) resulting in the poly(2-methyl-2-oxazoline)$_{50}$-*block*-(2-phenyl-2-oxazoline)$_{50}$ block copolymer. The final work-up of the polymer was performed by precipitation in tetrahydrofuran allowing complete removal of sulfolane. Approximate yield, 70 %.

Synthesis of defined 50,000 g/mol poly(2-methyl-2-oxazoline)

For the synthesis of the defined 50,000 g/mol PMeOx polymer the sacrificial initiator method (SIM) was used according to a recently filed patent procedure by Monnery *et al.* (*vide* **chapter 4**, for a detailed description).[39] Shortly it describes the polymerization of 2-oxazolines *via*

purification of the MeOx and sulfolane over a sacrificial amount of initiator, being a 2-phenyl-2-oxazolinium tetrafluoroborate salt (HPhOx-BF_4). After the purification step, a static distillation is performed of the solvent and the remaining monomer towards a polymerization flask in which the amount of initiator needed for the polymerization reaction is already present. For the polymerization mixture comprising of 45 mL MeOx (3 M) and 131 mL sulfolane, 1.24 g of HPhOx-BF_4 initiator was used to purify the reaction mixture *in situ*. In the polymerization flask 151 mg of HPhOx-BF_4 (3.6 mM) was added to obtain a PMeOx polymer with a target DP of 823 (target M_n 70,000 g/mol at 100 % conversion). In order to obtain a 50,000 PMeOx polymer, 70 % conversion is targeted. Therefore, the reaction mixture is put in an oil bath at 60 °C, for 75 hours. After the reaction, 4 mL of a methanolic KOH solution (1 M) was added. The purification is performed by precipitation of the polymer from the reaction mixture in cold diethyl ether followed by a second precipitation in tetrahydrofuran (THF). Final drying is performed by dissolution in water and consequent freeze drying of the solution to obtain the PMeOx as a white powder.

2.3 RESULTS AND DISCUSSION

2.3.1 Solvent screening for the CROP of 2-methyl-2-oxazoline

In the search for more polar solvents for the CROP of MeOx, we initially proposed *N,N*-dimethyl acetamide (DMA) and sulfolane. DMA has been identified as solvent according to the similarity towards the tertiary amide backbone of the PMeOx structure. Sulfolane was chosen as an alternative dipolar aprotic solvent, which is mostly used as an industrial solvent but recently also used as a universal solvent for ATRP.[36] Apart from the extremely high dipole moment (4.7 D), high boiling point and high chemical stability, sulfolane has a high solvency power for cations (*i.e.* high Hildebrand solubility parameter), which is expected to be favorable for the CROP of 2-oxazolines.[35] In both DMA and sulfolane, MeOx and PMeOx are found to be soluble[i] (**Table 3**), making them promising candidates for the CROP of MeOx. Furthermore, sulfolane is regarded as class II solvent, which has to be limited for human consumption but not completely avoided.[39]

After the solvent solubility screening, a series of kinetic studies was performed for the CROP of MeOx with a target DP of 100 in DMA and sulfolane at 140 °C under microwave conditions, in order to compare the results with the conventional polymerization of MeOx in CH_3CN. Note that

[i] The PMeOx solublity is based on the visual determination of the homogeneity of the polymerization mixture after termination. Insolubility of PMeOx in *e.g.* chlorobenzene is based on the cloudiness of the polymerization mixture. The exact solubility is not determined, because of the difficulty to solubilize these high polymerization concentrations of the polymer in a particular solvent.

PMeOx with a DP of 100 is still soluble in CH_3CN enabling the kinetic study, but higher DP PMeOx is no longer well-soluble in CH_3CN. All polymerizations showed linear first-order kinetics (**Figure 2**).

Table 3. Overview of the tested polymerization solvents with corresponding dipole moments, Hildebrand parameters and solubility of PMeOx in each solvent.

Solvent	Dipole moment [35]	Hildebrand solubility[35]	P(MeOx) solubility[a]
Chlorobenzene	1.54	9.5	-
DMA	3.73	23.3	+
CH_3CN	3.44	11.9	+/-
Sulfolane	4.69	27.2	++
MeOx	n.d.[b]	n.d.[b]	-

[a]Experimentally determined; [b]not determined

Remarkably, the microwave polymerization in sulfolane revealed a 3-fold increase of the k_p (k_p = 393*10^{-3} L mol^{-1}s^{-1}) compared to the polymerization in CH_3CN (k_p = 133*10^{-3} L mol^{-1}s^{-1}) and DMA (k_p = 103*10^{-3} L mol^{-1}s^{-1}). The size exclusion chromatography (SEC) traces of the final polymers reveal that DMA offers rather poor control over the CROP of MeOx, most likely due to trace impurities, that are very difficult to remove from DMA, whereas the SEC traces for the polymers obtained in CH_3CN and sulfolane reveal quite well-defined PMeOx with Đ values lower than 1.2.

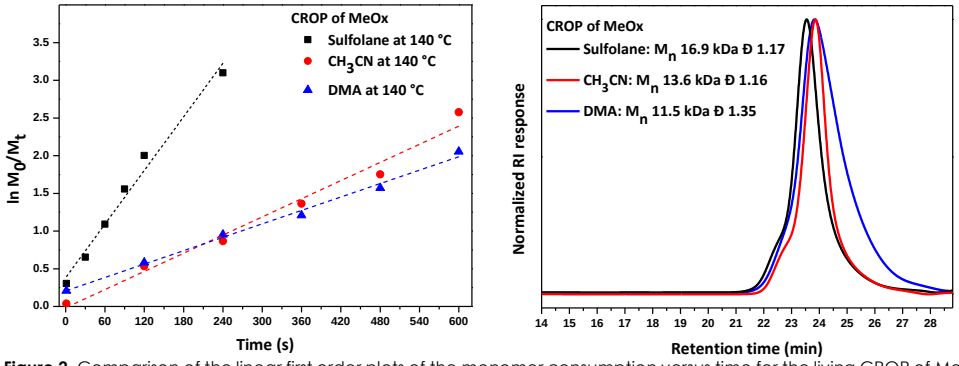

Figure 2. Comparison of the linear first order plots of the monomer consumption versus time for the living CROP of MeOx in sulfolane with MeOTs as initiator, at 140 °C with a DP of 100 (**left**); corresponding SEC-traces with DMA/LiCl as SEC eluent (**right**).

2.3.2 *Kinetic studies at different temperatures for the CROP of 2-methyl-2-oxazoline, 2-ethyl-2-oxazoline and 2-phenyl-2-oxazoline in sulfolane*

In order to gain further insights in the CROP of MeOx in sulfolane, the CROP was investigated at different temperatures, *i.e.* 60 °C; 80 °C; 100 °C; 120 °C and 140 °C (**Figure 3A**). From the Arrhenius plot (ln k_p vs 1/T) the respective Arrhenius parameters with regard to equation (2) could

be derived, revealing an activation energy (E_a) of 81.1 kJ/mol in sulfolane, which is in the range of the reported E_a for the CROP of MeOx in CH_3CN (Wiesbrock et al., E_a = 75.4 kJ/mol). The frequency factor (A), however, shows a large increase, with a value of 63.96 x 10^8 s^{-1} for the CROP of MeOx in sulfolane versus 5.05 x 10^8 s^{-1} for the CROP of MeOx in CH_3CN.

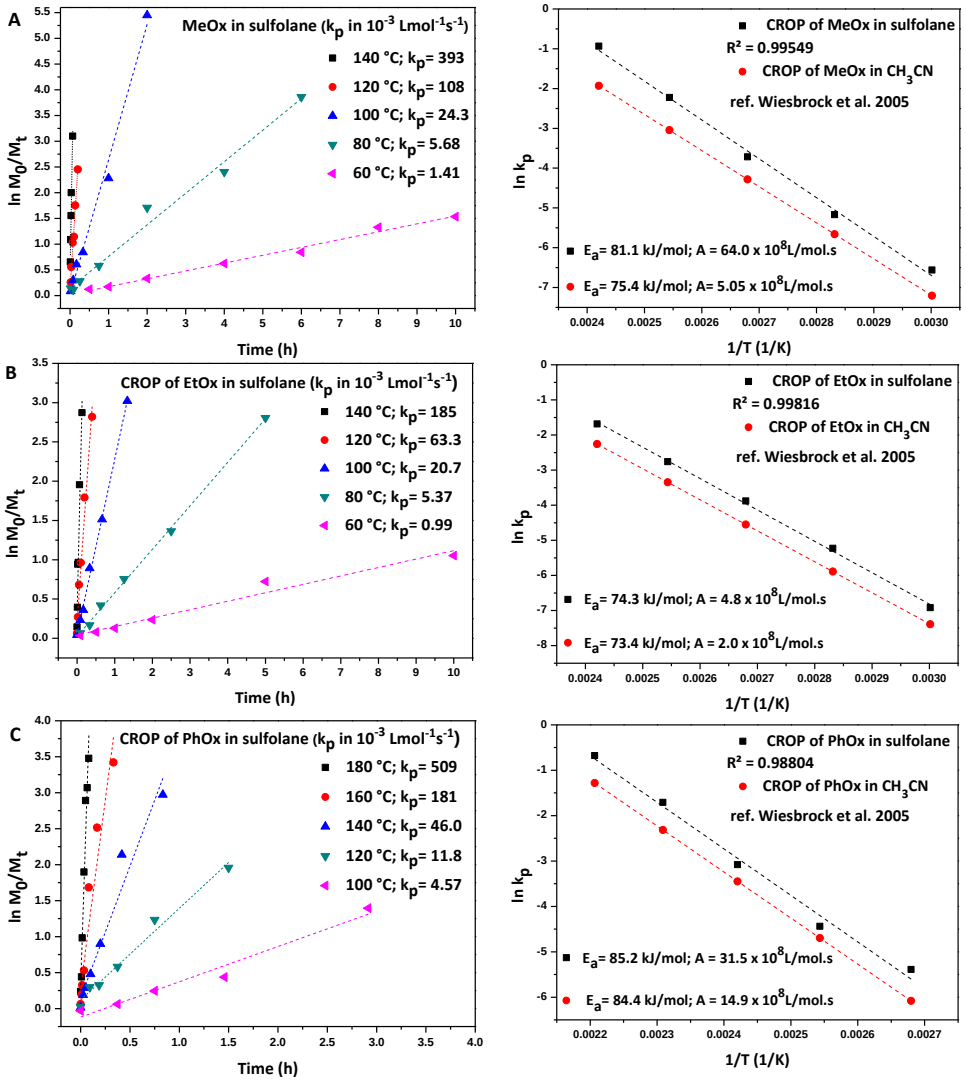

Figure 3. First-order kinetic plots, for the monomer consumption as a function of time for the cationic ring-opening polymerization of MeOx (**A**), EtOx (**B**) and PhOx (**C**) in sulfolane at 3 M monomer concentration with MeOTs as initiator and target DP of 100. (**left**) Since the kinetic plots are determined at different temperatures for each monomer in sulfolane, the corresponding Arrhenius plots could be determined; as a reference the Arrhenius plots for the polymerization of the corresponding monomers in CH3CN are also shown.[12] (**right**)

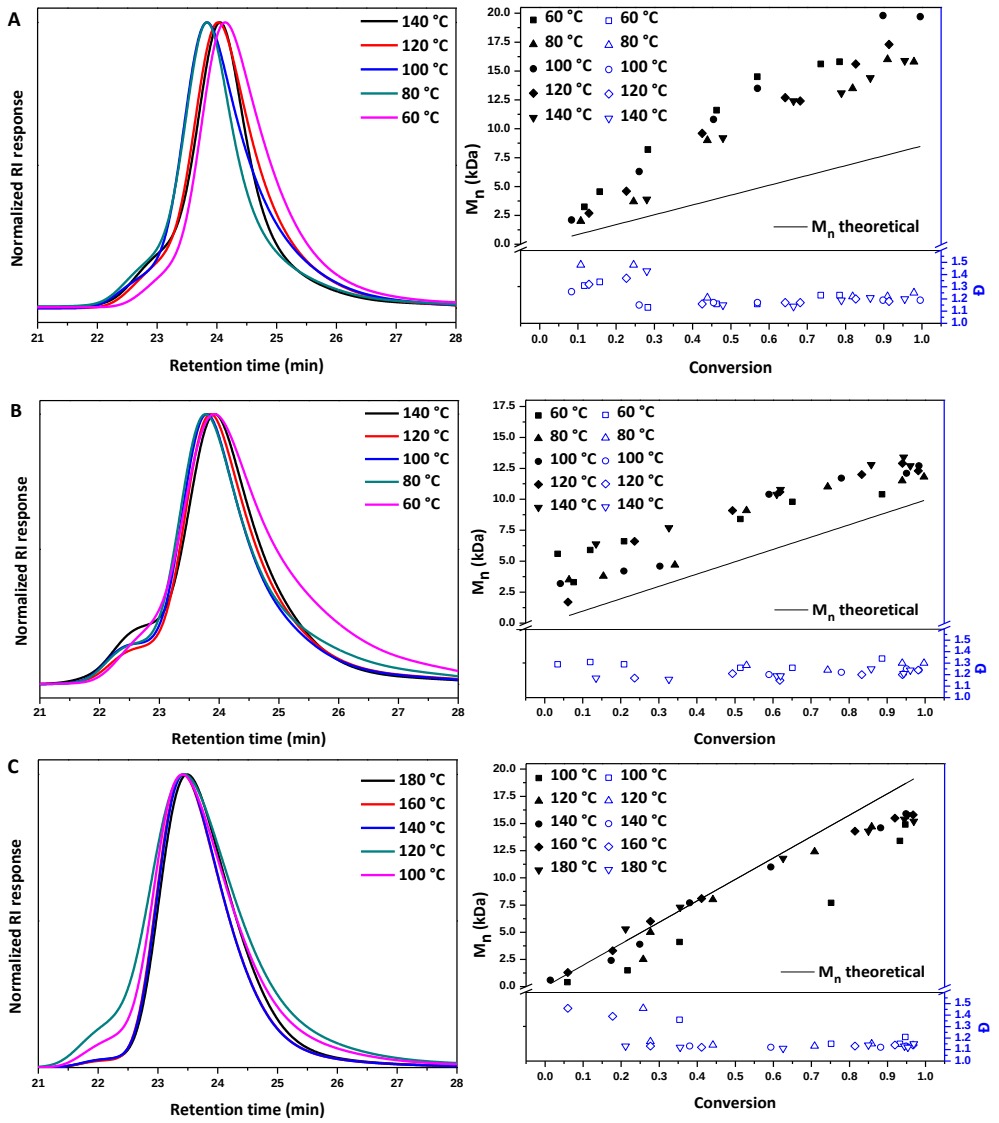

Figure 4. Size-exclusion chromatography traces for PMeOx (**A**), PEtOx (**B**) and PPhOx (**C**) polymers obtained in sulfolane. For good comparison and simplification of the results from the kinetic studies, all the samples with conversion over 90 %, at the different temperatures, are shown. (**left**) The relationship between the dispersity and the experimental and theoretical number average molecular weight *versus* the conversion is plotted for the CROP of MeOx, EtOx and PhOx. (**right**)

The frequency factor, A, can be interpreted as the fraction of collisions that possess enough kinetic energy for the reaction to proceed. Therefore, the experimental observations mean that the CROP of MeOx in sulfolane leads to a higher frequency of effective collisions than the CROP of MeOx in CH_3CN. A possible explanation for this high 'A' value can be found in the higher polarity and dielectric constant of sulfolane in comparison to CH_3CN, which may result in higher rotational energy of the sulfolane molecules and may eventually lead to a higher rate of successful collisions. Finally, this leads to a higher k_p value for the CROP of MeOx in sulfolane as the rate of successful collisions, i.e. the product of A and the E_a term, is defined as the k_p.

To extend the scope of sulfolane as accelerating polymerization solvent for the CROP of 2-oxazolines, kinetic studies for the CROP of EtOx (hydrophilic) and PhOx (aromatic and hydrofobic) were also performed at different temperatures (**Figure 3 B-C**). Both the k_p's for the CROP of EtOx ($k_p^{140\,°C}$ = 185 x 10^{-3} L $mol^{-1}s^{-1}$) and PhOx ($k_p^{140\,°C}$ = 46 x 10^{-3} L $mol^{-1}s^{-1}$) at 140 °C in sulfolane revealed faster polymerizations compared to the CROP of the respective monomers in CH_3CN, *i.e.* EtOx ($k_p^{140\,°C}$ = 104 x 10^{-3} L $mol^{-1}s^{-1}$) and PhOx ($k_p^{140\,°C}$ = 32 x 10^{-3} L $mol^{-1}s^{-1}$). Although the polymerization solvent has been changed from CH_3CN to sulfolane, the order of k_p remains unchanged with the CROP of MeOx being faster than EtOx, followed by PhOx. However, it is remarkable that the acceleration of the CROP of MeOx is significantly higher than for the EtOx and PhOx polymerizations in sulfolane, compared to CH_3CN (**Table 4**). This observation can be explained by the higher solvent compatibility (in terms of polarity) of MeOx for sulfolane, compared to EtOx and PhOx. From the Arrhenius plots (**Figure 3**, **Table 2** experimental) it can be concluded that for all the 2-oxazoline monomers the E_a for their CROP in sulfolane remains more or less unchanged compared to the E_a in CH_3CN. On the contrary, the CROP of 2-oxazolines show a higher A value in sulfolane compared to the CROP in CH_3CN, which demonstrates clearly the solvent induced acceleration by sulfolane.

Table 4. Comparison of the propagation rate constants (k_p) at 140 °C in sulfolane and CH_3CN, including acceleration ratios.

Monomer	Solvent	K_p (140 °C)	Acceleration
		L $mol^{-1}s^{-1}$	**Ratio ($k_p/k_{p,CH_3CN}$)**
MeOx	sulfolane	393	2.95
MeOx	CH_3CN	133	-
EtOx	sulfolane	185	1.78
EtOx	CH_3CN	104	-
PhOx	sulfolane	46	1.44
PhOx	CH_3CN	32	-

DMA-SEC analysis demonstrated that all synthesized PAOx polymers have a Đ of 1.1 to1.3, which is similar to the previous reported results for the CROP of 2-oxazolines in CH_3CN (**figure 4**).[35] A slight increase in the Đ is observed when lowering the polymerization temperature, which could be an indication of slower initiation. Furthermore, the linear relationship between the degree of conversion and the experimental M_n demonstrates the livingness and controlled character of the CROP of MeOx, EtOx and PhOx. It is important to note that the experimental M_n values are calculated against polymethylmethacrylate standards. Therefore, the experimental M_n values are overestimated for the hydrophilic polymers PMeOx and PEtOx and underestimated for the hydrophobic polymer PPhOx (**Figure 4**).

2.3.3 *Synthesis of a poly(2-methyl-2-oxazoline)-block-(2-phenyl-2-oxazoline) copolymer*

As a final proof for the livingness of the CROP of 2-oxazolines in sulfolane, a block copolymer has been synthesized consisting of a first block of PMeOx followed by a second block of PPhOx. Here, the PMeOx block was synthesized first, to assure fast initiation of the second PPhOx block after sequential addition of the PhOx monomer. The SEC analysis (**Figure 5**) shows the first PMeOx block, sampled before addition of the second monomer, and the resulting PMeOx-block-PPhOx copolymer, with Đ values of 1.1. The NMR analysis further confirmed the desired structure. Importantly, the prepared diblock copolymer has a significantly lower Đ than the previously reported analogue that was prepared in CH_3CN (Đ = 1.25), which may be a result of improved solvent compatibility of both the hydrophilic (PMeOx) and hydrophobic blocks (PPhOx).[12]

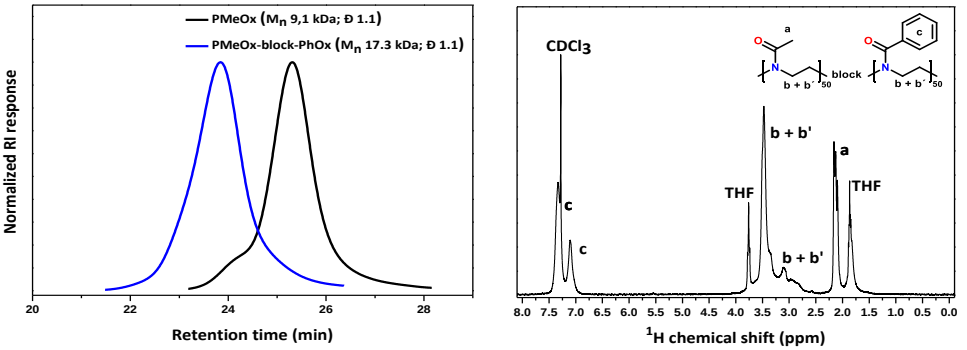

Figure 5. Size exclusion chromatography trace of first $PMeOx_{50}$ block and resulting $PMeOx_{50}$-block-$PPhOx_{50}$ copolymer. (**left**) Corresponding NMR analysis of block copolymer after precipitation in tetrahydrofuran. (**right**)

2.3.4 *Synthesis of high molecular weight poly(2-methyl-2-oxazoline)*

In a final attempt to demonstrate the importance of sulfolane as solvent, it is shown that the polymerization of a 50,000 g/mol PMeOx polymer can be achieved in sulfolane as polymerization solvent. The reaction mixture is purified according to the SIM (*vide supra* **section 2.2.2**) and performed at 60 °C for 75 hours. After purification of the prepared PMeOx by precipitation and freeze drying, the characterization results from the size-exclusion chromatogram in **Figure 6** show an M_n of 45,000 g/mol, M_p of 63,600 g/mol and a relatively high Đ value of 1.4. The SEC trace indicates non-ideal conditions for SEC measurements with this HPLC system set-up; as the extremely hydrophilic PMeOx polymer might show atypical band-broadening and tailing to low molar mass which might be the result of column interactions. The fact that low molar mass tailing is observed without a higher molar mass coupling shoulder indicates that the tailing is likely not due to chain-transfer side reactions, although these cannot fully be excluded. Nevertheless, the experiment proved the ability of sulfolane to act as a solvent to homogeneously prepare high molar mass PMeOx polymers in a relatively defined manner.

Although the synthesis of this 50,000 g/mol PMeOx polymer has succeeded, there is still room for improvement, in terms of obtaining a defined polymer or improving the SEC analysis. Improvements can be done *e.g.* by purifying sulfolane over sacrificial initiator every time prior to polymerization, separately from the MeOx monomer. In this way higher temperatures can be used for its purification, without doing any harm to the monomer. Also the search for other solvents can still continue in future research.

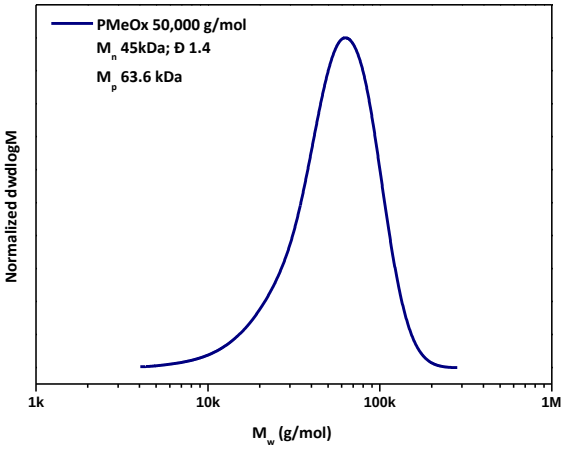

Figure 6. Size-exclusion chromatography result for a 50,000 g/mol PMeOx polymer, in *N,N*-dimethyl acetamide against PMMA standards.

2.4 CONCLUSIONS AND OUTLOOK

In conclusion, it is clearly shown that sulfolane can be used as a polymerization solvent for the CROP of hydrophilic and aromatic (hydrophobic) 2-oxazoline monomers, such as MeOx, EtOx and PhOx. The overall polymerization rate is found to be faster in sulfolane, compared to the standard polymerization solvent CH_3CN, which opens tremendous possibilities towards further optimization of the CROP of 2-oxazolines with regard to decreasing reaction times for the synthesis of PAOx, albeit at the cost of more complicated solvent purification and removal. If one compares the Arrhenius parameters for the CROP of 2-oxazolines in sulfolane compared to acetonitrile, the E_a is found to be similar but the frequency factor is significantly larger. In future research, the synthesis of high molar mass PMeOx will be targeted and investigated, which is facilitated by the good solubility of PMeOx in sulfolane, enabling further use of PMeOx in biomedical applications. Finally, the fact that the acceleration of the MeOx, EtOx and PhOx monomers are relatively different compared to the polymerization in CH_3CN, further formation of alternative and stronger gradient copolymers can be explored by changing only the solvent.

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Chapter 3

CHAPTER 3

3 SOLVENT OPTIMIZATION FOR THE CATIONIC RING-OPENING POLYMERIZATION OF 2-ETHYL-2-OXAZOLINE

ABSTRACT Insights in the polymerization of 2-ethyl-2-oxazoline (EtOx) are of extreme importance since PEtOx is the most common poly(2-oxazoline) polymer for biomedical applications resulting from its high water-solubility, good monomer availability, relatively easy CROP as well as its widespread comparison in literature studies against poly(ethylene glycol) (PEG), which is regarded as the golden standard in this field. In this chapter different solvents were investigated to challenge the current state of the art polymerization of EtOx in acetonitrile in order to check the influence of the polymerization solvent on the reaction kinetics, the polymer structure in terms of molar mass distribution (*i.e.* dispersity, Đ) and the higher molecular weight (MW) polymer synthesis. Both overall reaction speed and green synthesis methods were investigated to strengthen the case of PEtOx used as a biomedical friendly polymer. Furthermore, optimized methods for the synthesis of both defined low and high molecular weight PEtOx are proposed, according to trends seen in extended kinetic studies for a series of solvents bearing different functional groups.

Contributors to this work

Bart Verbraeken and Bryn D Monnery contributed to the ideas for the selection of solvents and discussion of the data.

Contribution of the candidate

Performed all experiments in combination with analysis and discussion of the data.

Vergaelen, M *et al.*, The use of a green solvent for the synthesis towards poyl(2-ethyl-2-oxazoline), a breakthrough for biomedical applications. To be submitted in Green Chemistry.

Chapter 3

3.1 STATE OF THE ART: THE CATIONIC RING-OPENING POLYMERIZATION OF 2-ETHYL-2-OXAZOLINE

Up to now, poly(2-ethyl-2-oxazoline) (PEtOx) is the only PAOx polymer that is commercially available on ton scale by Polymer chemistry innovations (PCI). This commercial grade is called Aquazol® and consists of ill-defined (Đ ~ 3-4) PEtOx with a broad range of molecular weights (MW). Aquazol® is produced *via* a bulk polymerization process, explaining these poor molar mass distribution characteristics and poor batch-to-batch reproducibility. Furthermore, Aquazol® is mainly used for large scale applications such as paint fillers, lubricants and adhesives.[1] Its exceptional water solubility and thermal processing capability makes it an excellent alternative for polymers like poly(vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVOH) as stated by PCI. Despite the commercial availability of Aquazol®, it is not suitable for pharmaceutical use, because of the poorly controlled production process and solvent residues. Recently, also defined PEtOx has been made commercially available on kg scale by Ultroxa®, a spin-off company by Hoogenboom *et al*.[2]

The last decades, PEtOx has gotten most attention in the field of biomedical and pharmaceutical applications (as explained in **chapter 1**).[3–6] In the literature, many examples can be found from its use in, for example polymer-prodrug conjugates, hydrogels and nanofibers.[7–10] Besides those examples, PEtOx can also undergo acidic (or basic) hydrolysis of the side chains, leading to linear poly(ethylene imine) (PEI). At present, PEtOx is still the preferred precursor for the synthesis of PEI, which is mostly known as transfection agent for DNA delivery.[11–13] Additionally, PEtOx-EI statistical copolymers can be prepared by partial hydrolysis of PEtOx, which can be utilized for post-polymerization modification.[14,15]

Furthermore, if we consider the cationic ring-opening polymerization (CROP) and its mechanism, PEtOx is relatively easy to synthesize compared to PMeOx (*vide* **chapter 2**), which is more prone to chain transfer and coupling reactions. Another drawback for PMeOx, is its poor solubility in a wide variety of organic solvents, which makes it difficult to control its polymerization and to do post-polymerization modifications. Conversely, 2-ethyl-2-oxazoline and the resulting PEtOx polymer are easily solubilized in a series of polar and even apolar solvents, which is an important asset to keep control over the CROP of 2-oxazolines. This makes it very interesting to investigate the CROP of EtOx for the preparation of PEtOx, which is illustrated by the numerous literature articles and reviews on this topic. In first instance different initiator systems have been studied using EtOx as standard monomer, including alkyl sulfonates, such as methyl *p*-toluenesulfonate (MeOTs), which is most frequently found in literature *p*-nitrobenzenesulfonates (nosylates) and trifluoromethanesulfonates (triflates), alkyl, benzyl and

acetyl halides, oxazolinium salts and lewis acids.[16–21] Secondly a large variety of terminating groups, including all possible nucleophiles, have been investigated revealing a dual mechanism of termination on respectively the 5 position, for strong nucleophiles, and the 2 position for weaker nucleophiles and water.[22,23] Besides these chain-end functionalizations, several studies show accelerated copolymerization behavior with EtOx as reference comonomer.[24–26] Nevertheless, a broad comparative on different polymerization solvents, to look at the effects of ion-pairing, cationic versus covalent oxazolinium species, viscosity and polymerization speed, for the CROP of EtOx, has never been performed.

Key literature examples on the effect of solvents on the CROP of 2-oxazolines include the evaluation of solvents bearing different functional groups, as published by Litt *et al.* in the late 60s,[27] the CROP of MeOx by Dworak[28] and the low temperature polymerization of 2-isopropyl-2-oxazoline in chlorobenzene, published by Monnery *et al.*[29] Up to date most of the kinetic studies on the CROP of 2-oxazolines were limited to different monomers in a common organic solvent, *e.g.* in acetonitrile, but we are not aware of a broad solvent screening for the polymerization kinetics of the CROP of EtOx at different temperatures . Most probably because the combination of either the use of acetonitrile, for high temperature polymerizations of short PEtOx polymers and chlorobenzene for the synthesis of high MW PEtOx at low temperature, serves the needs for the preparation of PEtOx. Despite that these solvents work well, it may be interesting to challenge them with other solvents to find out if other options turn out to outperform these findings.

In this chapter first the kinetics of the CROP of EtOx are investigated in a series of solvents such as acetonitrile,[30,31] chlorobenzene, anisole, nitrobenzene, sulfolane, 1,3-dimethyltetrahydropyrimidin-2(1H)-one (DMPU) and ethyl acetate (**Figure 1**). The choice for aromatic solvents was inspired by the successful polymerization of EtOx in chlorobenzene at low temperatures. Next to chlorobenzene, anisole and nitrobenzene were selected to study the influence of different electron withdrawing or donating substituents on the aromatic ring and the potential cation-π effect[i] on the overall polymerization speed. Since sulfolane as highly polar solvent showed increased reaction kinetics, DMPU was also selected as highly polar solvent to study the generality of the observations discussed in **chapter 2**. Ethyl acetate, was initially chosen to evaluate whether it can accelerate the CROP due to the electronic similarity with methyl ester bearing 2-oxazoline (MestOx) monomers. In a literature article, by Bouten *et al.*, it is described that the carbonyl moiety of the MestOx monomers stabilizes the oxazolinium chain-end during

[i] The cationic oxazolinium chain-end can be stabilized by π-electrons; increasing the electrophilicity on the other end of the oxazolinium ring (5-position); rate acceleration is noticed for the CROP of methyl ester bearing 2-oxazoline monomers.

propagation, thereby increasing the reactivity of the 5-position of the oxazolinium ring leading towards faster propagation.[32,33]

Finally, it is of utmost importance for biomedical applications that the synthesized defined PEtOx polymers, are safe to use and non-toxic. However, it is known that after the CROP of EtOx residues of the toxic monomer and solvents can remain in the final obtained PEtOx polymer. Moreover, the use of polymerization solvents such as chlorobenzene is deleterious for the environment. Therefore, the question is raised if ´a green method can be applied for the synthesis of defined (high MW) PEtOx polymers?'. In this regard, ethyl acetate may be considered as a green solvent.[34,35] If one looks at energy related costs for the environment, ethyl acetate is not ideal, but for health and safety related issues, ethyl acetate is one of the best non-harmful solvents together with small alcohols. If one looks at the specific application, to synthesize PEtOx polymers for the food and drug industry, ethyl acetate seems an ideal match for its production process, which will be further elaborated in the results and discussion part.

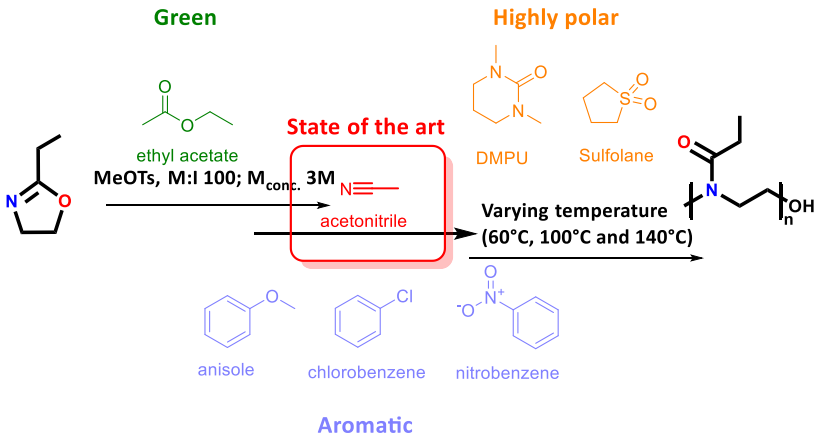
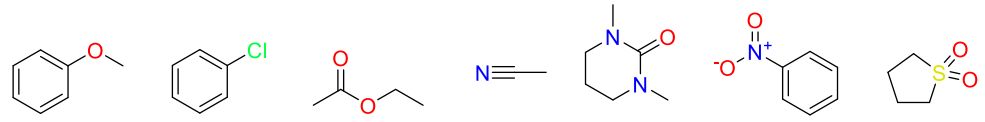


Figure 1. Proposed screening for the cationic ring-opening polymerization of 2-ethyl-2-oxazoline in different solvents.

Overview of the investigated solvents with their most important properties

The CROP of 2-oxazolines is commonly carried out in acetonitrile, which is a relative polar solvent compared to most common organic solvents. The polarity of acetonitrile enables fast and efficient heating in microwave reactions, *i.e.* also for the CROP of 2-oxazolines.[36–38] Even though acetonitrile is commonly used within PAOx polymerizations, it has disadvantages concerning side-reactions and impurities (extrinsic) (*vide* **chapter 1**, insights in the reaction mechanism). An overview of the different structures and properties related to all the different solvents used for the CROP of EtOx can be found in **Scheme 1** and **Table 1.**



Scheme 1. Overview of the different structures for the solvents used for the CROP of EtOx screening drawn with increasing polarity from left to right.

Table 1. Overview of the different characteristics, including dipole moment, the dielectric constant, the Hildebrand solubility parameter, the boiling point, the Dimroth solvent polarity, the viscosity and the molair volume; for the different solvents that were screened for the CROP of EtOx.[39–43]

	Dipole moment	Dielectric constant	Hildebrand solubility parameter	Boiling point	E_T (30) Dimroth	Viscosity (25 °C)	Molair volume
	D	-	δ	°C	Kcal mol^{-1}	cp	mL mol^{-1}
Anisole	1.38	4.33	9.9	154	37.1	0.778	109.2
Chlorobenzene	1.54	5.62	9.5	132	36.8	0.8	101.8
Ethyl acetate	1.88	6.02	9.1	77	38.1	0.45	97.8
Acetonitrile	3.44	37.5	11.9	82	45.6	0.38	52.5
DMPU	4.17	36.12	9.3	246	42.1	1.9	120.5
Nitrobenzene	4.28	34.8	10	211	41.2	1.8	102.3
Sulfolane	4.68	44	27.2	285	44	10.3	95.4

In this chapter, first an overview of the different properties, including polarity, viscosity, *etc.*, of the different solvents used for the screening of the CROP of EtOx will be given (**section 3.3.1**). In **section 3.3.2**, the kinetic data for the CROP of EtOx in different solvents at varying temperature regimes are discussed. Furthermore, the k_p´s are determined to achieve a direct comparison between the different solvents. Finally, a size-exclusion chromatography (SEC) analysis will be performed to confirm the conversions by MW and the controllability of the polymerization as a function of solvent. In **section 3.3.3**, the thermodynamics of the CROP of EtOx in terms of the solvents is examined by investigation and determination of the Arrhenius plot and corresponding parameters, as basis for interpretation of the observed kinetics in **section 3.3.2**. Lastly, in **section 3.3.4.**, the green polymerization in ethyl acetate is further investigated in terms of reproducibility and the synthesis of high MW PEtOx.

3.2 MATERIALS AND METHODS

3.2.1 Materials and equipment

Materials

Methyl *p*-toluenesulfonate (MeOTs), drying agents (barium oxide, calcium hydride, sodium pallets), potassium hydroxide and solvents (sulfolane, acetonitrile (CH_3CN), chlorobenzene, anisole, nitrobenzene, DMPU and ethyl acetate) (HPLC quality or Acroseal®) were purchased from both Sigma-Aldrich and Thermofisher/Acros organics. 2-Ethyl-2-oxazoline (EtOx) was kindly donated by PCI. CH_3CN was purified over aluminum oxide by means of a solvent purification system from J.C. Meyer.

Purification methods

EtOx was distilled over barium oxide under inert argon atmosphere before its use in the polymerizations. **MeOTs** was distilled over CaH_2 under reduced pressure. Purification of **sulfolane** was performed according to the procedure described in the book 'Purification of Laboratory Chemicals', 6th edition.[44] Initially, small portions of solid $KMnO_4$ were added to the sulfolane (at 50 °C) until the color remains up to one hour. This is mainly to deal with 3-sulfolene and 2-sulfolene impurities. Thereafter, a dropwise addition of MeOH is performed to destroy the excess of $KMnO_4$ followed by filtering the solution. Note: to clean the filter afterwards (MnO_2 is quite harsh to remove) concentrated sulfuric acid was used. Trace amounts of MeOH were removed on the rotavap for few a minutes. Then CaH_2 was added as a drying agent and the mixture was stirred overnight. Afterwards a distillation was carried out under high vacuum (hot plate > 90 °C and 10^{-2} mbar), without any condenser (sulfolane becomes a solid at ca. 30 °C). The whole distillation set-up was covered in aluminum foil to increase the heat transfer and only the receiving flask was cooled with ice. For the **purification of chlorobenzene**, first a washing step with sulfuric acid is performed. To 500 mL chlorobenzene, 60 mL sulfuric acid is added. The mixture is stirred at 850 rpm for 30 min. Afterwards a liquid-liquid separation is performed, with the chlorobenzene as upper phase and sulfuric acid as lower phase. This procedure is repeated until the sulfuric acid phase remains colorless, which is typically the case after three washing steps. The chlorobenzene is then washed three times with 50 mL of an aqueous solution of sodium bicarbonate (13.05 g in 150 mL water). Between each washing step, the solution is stirred for 30 min at 1000 rpm. During separation, the chlorobenzene is at the lower phase with the aqueous upper phase. During the first washing step some heavy bubbling is observed, due to the formation of carbon dioxide. Finally, the chlorobenzene is washed three times with (distilled) water. Between each step the

mixture is again stirred for 30 min at 1000 rpm. After these washing steps, the chlorobenzene is dried over magnesium sulphate, which is followed by filtration and an additional drying step over barium oxide is performed. This solution is stirred overnight at 60°C at 850 rpm. The final distillation to dryness is performed under argon overpressure. **Anisole, DMPU and nitrobenzene** were all dried over barium oxide and fractionally distilled. The center cut was then distilled again over barium oxide. **Ethyl acetate** was purchased from Fisher Scientific in an anhydrous grade and used as received.

Equipment

Conversions for the CROP of the selected 2-oxazoline monomers were monitored by gas chromatography (GC) analysis. GC was performed on an Agilent 7890A system equipped with a VWR Carrier-160 hydrogen generator and an Agilent HP-5 column of 30 m length and 0.320 mm diameter. An FID detector was used and the inlet was set to 240 °C with a split injection of ratio 25:1. Hydrogen was used as carrier gas at a flow rate of 2 mL/min. The oven temperature was increased with 20 °C/min from 50 °C to 120 °C, followed by a ramp of 50 °C/min. to 240 °C.

All stock solutions and samples were prepared in a VIGOR Sci-Lab SG 1200/750 Glovebox System with an atmospheric water concentration below 0.1 ppm. For the polymerizations, a Biotage Initiator EXP Microwave System with Robot Sixty was used. During the polymerizations the microwave synthesizer operated at a constant set temperature which is monitored by the IR-sensor.

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50 °C equipped with two PLgel 5 µm mixed-D columns in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent is DMA containing 50mM of lithium chloride at an optimized flow rate of 0.5 mL/min. The spectra were analyzed using the Agilent ChemStation software with the GPC add on. Molar mass and dispersity (Đ) values were calculated against polymethylmethacrylate standards from PSS.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance 300 MHz spectrometer, at room temperature. The chemical shifts are given relative to trimethylsilane (TMS).

3.2.2 Experimental methods

Synthesis of Homopolymers

To perform the kinetic studies, 45 mL stock solutions have been made and divided over different 2.5 mL microwave vials, each filled with 700 µL to 800 µL of this stock solution. For each kinetic run at a certain temperature, 7 microwave vials have been filled and heated for a certain time. The stock solutions consist in general of a 3 M monomer (EtOx) concentration, 30 mM initiator (MeOTs) concentration and the polymerization solvents. All calculations have been done in order to synthesize polymers with a M:I ratio, *i.e.* a target degree of polymerization (DP), of 100 (**Table 2**).

Table 2. Calculations for the kinetic study for the homopolymerization of EtOx in diferent solvents (M= Monomer; I= Initiator; S = Solvent [ ] = concentration; V( ) = Volume).

Monomer	[M]/[I]	[M]	V (Stock, total)	[I]	V (I)	V (M)	V (S)
Name	Ratio	mol/L	mL	mmol/L	µL	mL	mL
EtOx	100	3	45	30	210	17.7	27.1

After the reaction, GC samples were made by sampling 100 µL of the reaction medium into a sample vial and adding 900 µL of chloroform, as non-interfering solvent. For the SEC analysis, the samples were terminated with 500 µL of a methanolic KOH solution, followed by 100 µL sampling of the reaction medium and addition of 900 µL of DMA. Additionally, all samples were filtered through 0.13 µm filters before SEC analysis (FE1322). In **Table 3** the resulting propagation rate constants (k_p) are listed.

Table 3. Summary of the kp's for the kinetic studies for the CROP of EtOx in a series of different solvents at different temperatures.

k_p (*10^3 L/mols)	60°C	100°C	140°C
Anisole	0.68 ± 0.03	13.05 ± 0.29	140.7 ± 6.6
Chlorobenzene	1.26 ± 0.06	19.66 ± 0.63	134.3 ± 4.8
Ethyl acetate	0.66 ± 0.03	11.56 ± 0.27	95.4 ± 1.5
Acetonitrile[36]	**0.57**	**9.91**	**99.1**
DMPU	0.63 ± 0.02	11.03 ± 0.51	133.0 ± 6.7
Nitrobenzene	0.79 ± 0.08	22.06 ± 0.67	211.3 ± 11.1
Sulfolane	0.99 ± 0.04	20.67 ± 0.49	185.3 ± 19.5

3.3 RESULTS AND DISCUSSION

3.3.1 Kinetic studies of the CROP of 2-ethyl-2-oxazoline in different solvents at varying temperatures

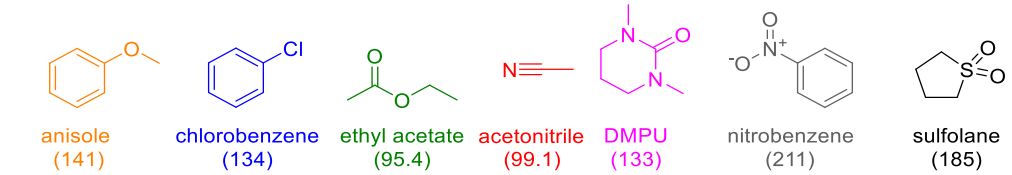
For the kinetic investigation on the CROP of EtOx a standard monomer to initiator (M:I) ratio, *i.e.* degree of polymerization (DP) of 100 repeating units, was chosen together with a 3 M monomer concentration. Furthermore, three temperatures were chosen, being 140 °C, 100°C and 60 °C. The high temperature region of 140 °C, is chosen to compare against the state-of-the-art synthesis of short PEtOx polymers in acetonitrile. At 140 °C, some solvents such as acetonitrile, chlorobenzene and ethyl acetate will exceed its boiling point, which might give an influence on the kinetics for the CROP of EtOx. The low temperature region of 60 °C, is chosen because high MW polymers are preferably made at lower temperatures, to limit side reactions (as explained in **chapter 1**). This is analogous to the low temperature polymerization in chlorobenzene.[29] In order to have a first idea on the Arrhenius parameters and thermodynamics of the CROP of EtOx in the different solvents, at least three different temperatures should be considered. Therefore, the middle temperature of 100 °C has been selected. It should be noted that for the kinetic studies for the CROP of EtOx in acetonitrile and sulfolane two additional temperatures (*i.e.* 80 and 120 °C) are included in the Arrhenius plot (**Figure 5**) based on the results of Wiesbrock *et al.* and chapter 2, respectively.

Some general conclusions can be drawn based on the first order kinetic plots retrieved from GC analysis and the MW data retrieved from SEC analysis. The details on the first order kinetic plots for the CROP of 2-oxazolines and how to extrapolate the k_p from the slope of the graphs is explained in **chapter 1**. Since the CROP is a controlled polymerization and the propagation should follow linear first order kinetics, the MW is also expected to be linear with conversion. This will be confirmed by SEC. In this section, the kinetics for the CROP of EtOx at the three different temperatures will be discussed separately (**Figure 2-4**).

Kinetic study of the CROP of EtOx at 140 °C

In the results (**Figure 2**) at 140 °C one can clearly see that the most polar solvents nitrobenzene and sulfolane induce the highest k_p and yield polymers with a slightly higher overall Đ in the CROP of EtOx. The k_p in DMPU, chlorobenzene and anisole is lower while the slowest CROP is observed in acetonitrile and ethyl acetate. This last observation might be related to the fact that the boiling point of the solvent is exceeded at 140 °C leading to pressurization of the reactions. Note that It is rather unexpected that DMPU does not follow the trend of acceleration

of nitrobenzene and sulfolane while it has only a slightly lower polarity than nitrobenzene. All solvents led to controlled polymerization as indicated by the linear increase of molar mass with conversion and the rather low Đ. Related to the molar mass distribution of the obtained polymer samples, the apolar solvents such as ethyl acetate, chlorobenzene and anisole, show lower Đ compared to the polar solvents sulfolane and nitrobenzene.



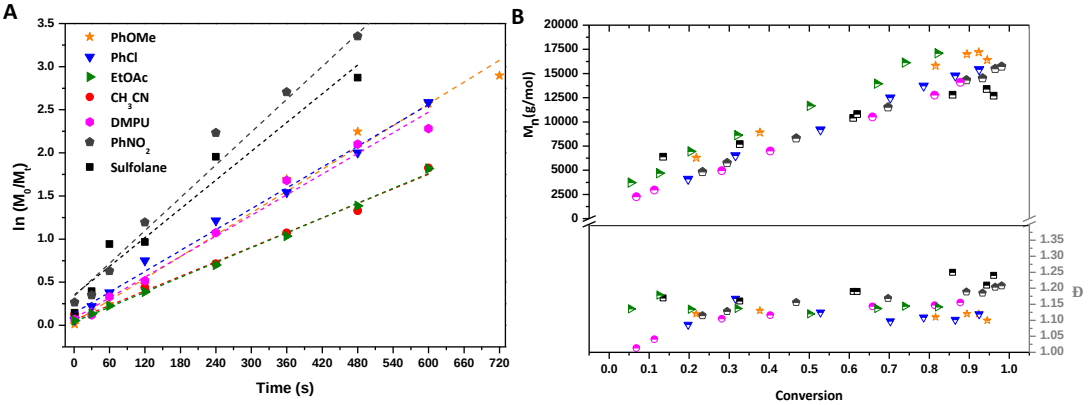


Figure 2. Kinetics for the CROP of EtOx in different solvents at 140 °C; beneath the solvent structures/names one can see the depicted values for the k_p at 140 °C in L/mol.s. **A)** first order kinetic plot; **B)** number average molecular weight M_n and dispersity (Đ) versus conversion plot.

Kinetic study of the CROP of EtOx at 100 °C

In the results (**Figure 3**) at 100 °C one can clearly see that the highly polar solvents like nitrobenzene and sulfolane still have the highest k_p. Remarkably, chlorobenzene now follows the trend of the highly polar solvents at 100 °C, which is under its boiling point. The other solvents including anisole, DMPU, ethyl acetate and acetonitrile (in respective order) follow similar trends a observed at 140 °C, within errors. It should be noted, however, that the CROP of EtOx in acetonitrile and ethyl acetate is relatively faster at 100 °C compared to 140 °C. Also at this lower polymerization temperature, the CROP is controlled as indicated by the linear increase of molar mass with conversion as well as the low Đ values. Related to the molar mass distribution of the obtained polymer samples, the apolar solvents, show lower Đ compared to the polar solvents.

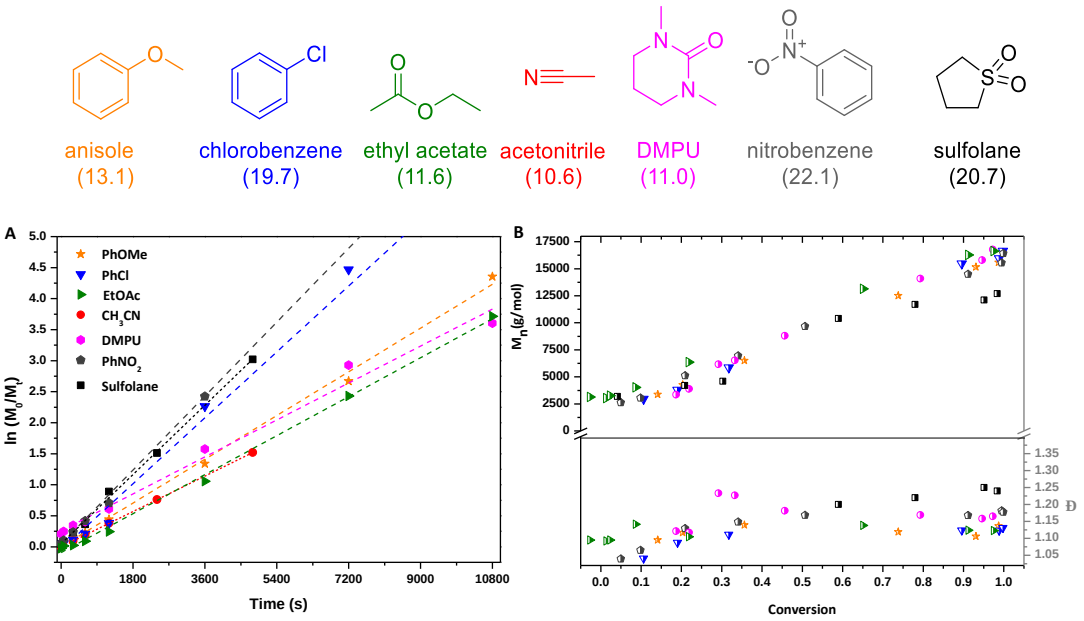


Figure 3. Kinetics for the CROP of EtOx in different solvents at 100 °C; beneath the solvent structures/names one can see the depicted values for the k_p at 100 °C in L/mol.s. **A)** first order kinetic plot; **B)** number average molecular weight M_n and dispersity (Đ) versus conversion plot.

Kinetic study of the CROP of EtOx at 60 °C

At 60 °C, the CROP of EtOx shows the highest k_p in chlorobenzene and sulfolane (**Figure 4**). The acceleration in chlorobenzene may be attributed to coordination and stabilization of the oxazolinium chain-end by cation-π interactions, thereby increasing the nucleophilicity of the C5 carbon on the oxazolinium ring (place of attack by subsequent monomers in the propagation step of the CROP of 2-oxazolines). The reason why the other aromatic solvents do not follow the same trend is unclear; a possible explanation for the slower polymerization in nitrobenzene could be related to the bulkiness of the nitro group as well as lower electron density together with potential coordination of the high electron density of the nitro group with the cationic oxazolinium moiety preventing cation-π interactions. For anisole, the lower polarity and inductive effect of the substituent, compared to chlorobenzene, may lower the k_p for the CROP of EtOx at 60 °C. The molar mass of the obtained polymers also increases with conversion at this temperature, but there is some scatter of the data and the final Đ values are higher compared to the other solvents indicating that the control over the polymerizations is somewhat less. Related to the molar mass distribution of the obtained polymer samples, the apolar solvents such

as ethyl acetate and chlorobenzene, with exception from anisole, show lower Đ compared to the polar solvents sulfolane, DMPU and acetonitrile hinting towards the occurrence of less side reactions in less polar solvents.

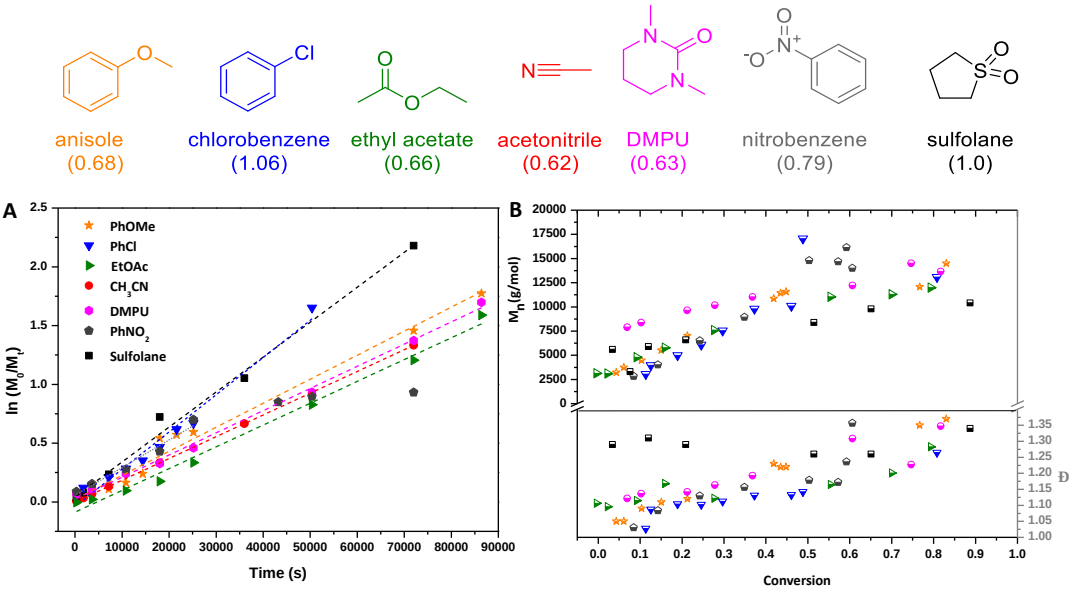


Figure 4. Kinetics for the CROP of EtOx in different solvents at 60 °C; beneath the solvent structures/names one can see the depicted values for the k_p at 60 °C in L/mol.s. A) first order kinetic plot; B) number average molecular weight M_n and dispersity (Đ) versus conversion plot. (Note: the linear fit for nitrobenzene has been made on the first five points)

3.3.2 Discussion on the Arrhenius parameters and trends for the polymerization of 2-ethyl-2-oxazoline in different solvents

Based on the kinetic study reported in 3.3.2, an Arrhenius plot was prepared by plotting the ln of the k_p's versus the inversed temperature. A linear correlation is expected (**Figure 5**) providing insights on the thermodynamics *via* the Arrhenius equation (**eq. 1-2**). One can determine the activation energy (E_a) from the slope and the Arrhenius frequency fractor (A) from the y-axis intercept of the Arrhenius plots for the given reactions in the different solvents.

$$k = A\, e^{\frac{-E_a}{RT}} \qquad \textbf{(1)}$$

$$\ln k = \ln A - \frac{E_a}{RT} \qquad \textbf{(2)}$$

The E_a represents the energy needed for the reaction to happen, being a successful collision. The Arrhenius frequency factor, A, gives the amount of successful collisions when the molecules have enough energy for the reaction to proceed.

First of all, if we look at **Figures 5-6 and Table 4** that list the Arrhenius plot/parameters and trends for the CROP of EtOx in the different solvents, it is difficult to generalize the conclusions based on these data as there is no direct correlation with *e.g.* polarity, dipole moment or dielectric constant of the solvents.

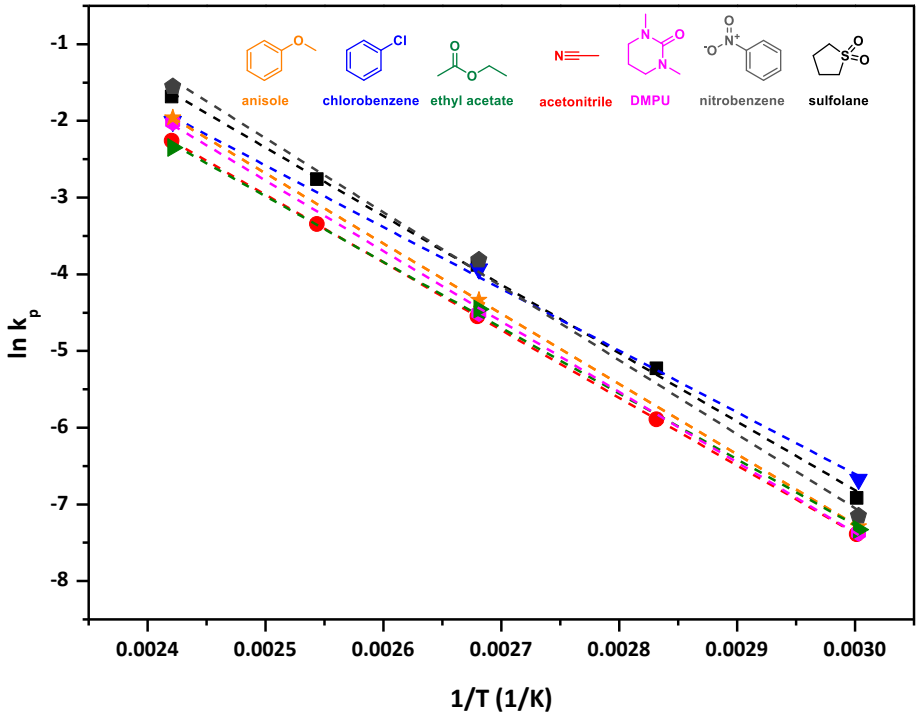


Figure 5. A) Arrhenius plot, giving the correlation of the ln of the propagation rate constant versus the inverse temperature. **B)** Zoom of the high temperature region at 140 °C. **C)** Zoom in the low temperature region, at 60 °C.

Nevertheless, some interesting observations are made. Based on the visualized trends of the k_p's as well as some characteristics as function of solvent in **Figure 6**, it appears that the k_p´s for the CROP of EtOx at the different temperature do best correlate with the boiling points of the different polymerization solvents. At this moment it is not clear why the k_p's correlate well with the boiling point of the solvents nor whether this is a just a coincidence rather than a real correlation. Another complication that obstructs hard conclusions on the correlation between the boiling points and the k_p´s is that in some cases the reaction temperature exceeds the boiling point of the particular solvent. Finally, a speculation about the boiling point is also complicated by the large variations in the MW of the utilized solvents that will also have some influence on the polymerizations. As a results, it is too early to make hard conclusions and more experiments would be required with different solvents.

Table 4. Overview of the Arrhenius parameters gathered from figure 5. for the CROP of EtOx in the different solvents. The values of the standard solvent, acetonitrile, are given as a reference value to interpret the other obtained values.

	E_a (kJ)	A (10^8)
Anisole	76.2 ± 0.1	6.09 ± 0.01
Chlorobenzene	66.9 ± 2.7	0.41 ± 0.02
Ethyl acetate	70.8 ± 3.0	0.89 ± 0.05
Acetonitrile[36]	**73.4 ± 0.5**	**1.99 ± 0.9**
DMPU	76.4 ± 1.6	5.90 ± 0.16
Nitrobenzene	80.2 ± 3.9	32.35 ± 1.89
Sulfolane	74.3 ± 1.6	4.79 ± 0.12

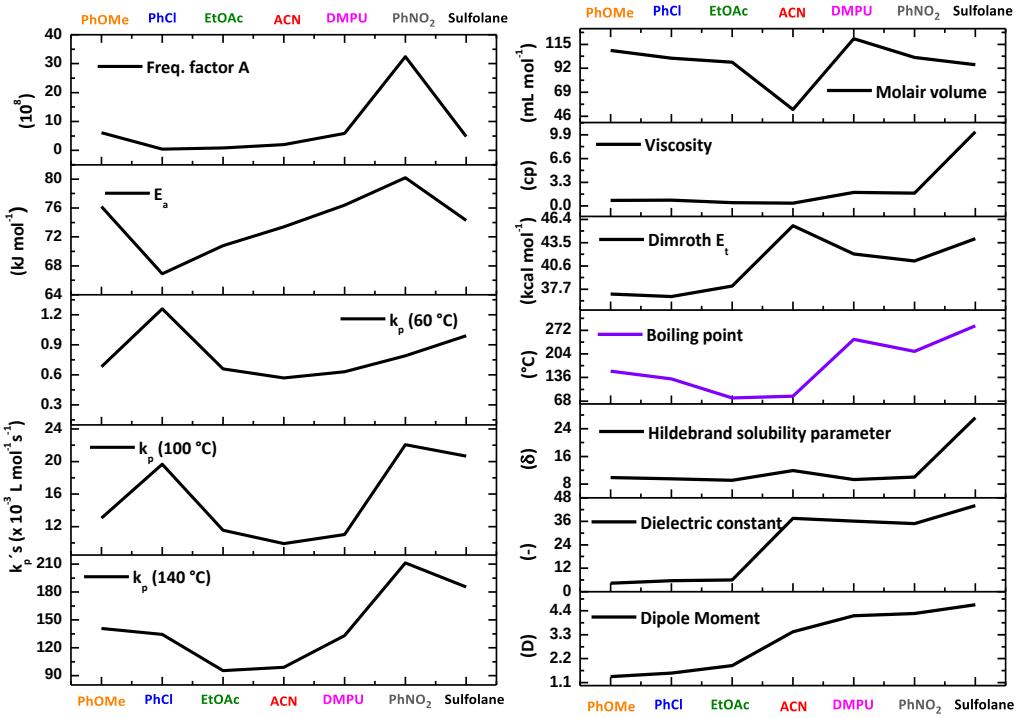


Figure 6. Overview of all the trends for k_p´s at 140 °C, 100 °C and 60 °C, and the Arrhenius parameters (E_a and freq. factor A) for the CROP of EtOx in the different solvents, sorted according to dipole moment. (**Left**) All the trends of the different parameters described in **Table 3** for the different solvents, which are sorted according to dipole moment. (**Right**)

The E_a and frequency factors for the CROP of EtOx in the different solvents (**Table 4**), summarizes the seen trends for the k_p´s in **section 3.3.2**. The highest E_a and frequency factor A is

seen for nitrobenzene, which explains the fastest CROP at 140 °C and the slowest at 60 °C. The complete opposite is observed for chlorobenzene, which has the lowest E_a and frequency factor A, explaining the relative increase in k_p towards lower polymerization temperatures. If we try to generalize the trends by any solvent parameter, it is shown that chlorobenzene is rather an exception than the rule, which may be related to the occurrence of specific cation-π interactions between the solvent and the oxazolinium chain ends.

3.3.3 Green synthesis method for the polymerization of 2-ethyl-2-oxazoline in ethyl acetate

Initial studies on the use of ethyl acetate as polymerization solvent for the CROP of EtOx, were performed to investigate potentially increased reaction rates, due to the electronic similarity of ethyl acetate with methyl ester bearing 2-oxazoline (MestOx) monomers. In a literature article, by Bouten *et al.*, it is described that the carbonyl moiety of the MestOx monomers stabilizes the oxazolinium chain-end during propagation, thereby increasing the reactivity of the 5-position of the oxazolinium ring leading towards faster propagation.[32,33] Consequently, we questioned if the carbonyl moiety of ethyl acetate will have the same influence on the propagation rate as observed for the MestOx monomers.

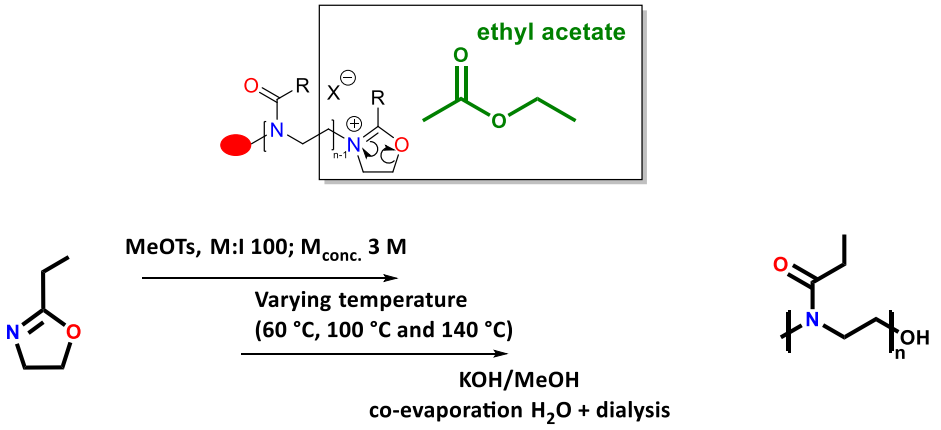
Additionally, it is of utmost importance for biomedical applications that the final product, *i.e.* the defined PEtOx polymers, are non-harmful and non-toxic for use in humans, provoking the question if *a green method can be applied for the synthesis of defined (high molecular weight) PEtOx polymers?* (**Figure 7**). In this regard, ethyl acetate can be considered as a green solvent, that is a much better candidate for environmental reasons compared to the state-of-the-art polymerization of EtOx in acetonitrile or chlorobenzene.

Jessop *et al.* and Capello *et al.* reported that it is crucial to stress the difference between on the one hand energy related costs for environment and on the other hand health and safety related issues which can both be accepted as 'green' chemistry concepts.[34,35] If one looks at energy related costs for the environment, ethyl acetate is not ideal. But for health and safety related issues, ethyl acetate is one of the best non-harmful organic solvents together with small alcohols, that are impossible to use as polymerization solvent for the CROP of 2-oxazolines due to their nucleophilicity. If one looks at the specific application - to synthesize PEtOx polymers for the food and drug industry - ethyl acetate seems to be an ideal match for its production process.

Up to date only one publication of C. Guerrero-Sanchez *et al.* mentions the efforts towards a green synthesis method for PEtOx polymers, using ionic liquids.[45] Here, the use of ethyl acetate

could also overcome the drawbacks related to using ionic liquids, including cost price, synthesis and purification.

At first, a test polymerization[ii] of EtOx in ethyl acetate was successfully performed, showing the conceptual idea of its use as polymerization solvent. In order to verify its further value, a full kinetic study on the CROP of EtOx in ethyl acetate is completed at different temperatures (60 °C, 100 °C and 140 °C; **Figure 8A**) under microwave irradiated conditions, revealing linear polymerization kinetics as expected for the CROP of EtOx in absence of termination reactions. It should be noted that the reaction kinetics were performed in triplicate, to both show reproducibility and statistical errors on the measured polymerization data. This means that every data point consists of three different reactions (out of three different stock



solutions) and every reaction is measured at a certain temperature for at 8 different time points, which gives in total 72 different polymerization reactions.

Figure 7. Investigated reaction conditions for the green synthesis of poly(2-ethyl-2-oxazoline).

The monomer consumption versus time at different temperatures is plotted in a linear first order kinetic plot in **Figure 8A**, together with the k_p´s. Furthermore, there is a linear correlation between the M_n and the monomer conversion which shows control over the MW for the CROP of EtOx in ethyl acetate indicative of the absence of significant chain transfer reactions (**Figure 7B**). Finally, in **Table 5** the k_p´s of both the CROP in ethyl acetate and acetonitrile are listed, for their direct comparison.

[ii] A test polymerization consists of the synthesis of a DP 100 PEtOx polymer under standard microwave conditions used for the CROP of EtOx in acetonitrile, *i.e.* 140 °C, MeOTs as initiator, 3 M monomer concentration, 12 min reaction time and ethyl acetate. SEC analysis of the PEtOx polymer from the test polymerization should give a Đ value below 1.2, to be successful.

Table 5. Propagation rate constants (k_p) for the CROP of EtOx in ethyl acetate and acetonitrile.

CROP of EtOx	Ethyl acetate	Acetonitrile[46]
k_p	x 10^{-3} L $mol^{-1}s^{-1}$	x 10^{-3} L $mol^{-1}s^{-1}$
140°C	95.4 ± 1.50	99.1
100°C	11.6 ± 0.269	9.91
60°C	0.66 ± 0,0267	0.57

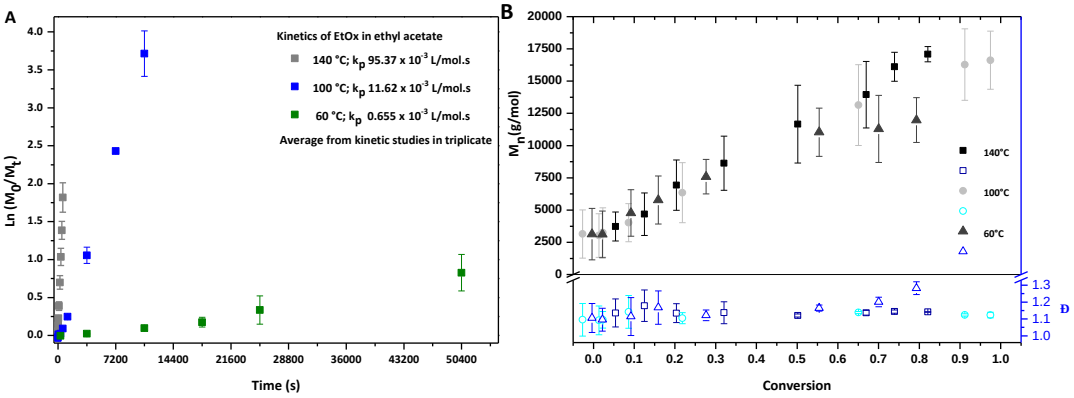


Figure 8. A) First order kinetic plot for the CROP of EtOx, showing linear reaction kinetics for the propagation. **B)** Number average molecular weight (M_n) and molar mass distribution, as dispersity (Đ) versus conversion plot, showing linear correlation indicative of control over molecular weight. All size-exclusion chromatography results for these plots were calculated against PMMA standards, for good comparison to previous obtained results in literature for the polymerization of EtOx in acetonitrile.

Since the kinetic study is performed at three different temperatures, an Arrhenius plot can be made giving insights in the polymerization thermodynamics (**Figure 9A**). As stated before, with the results from the kinetic study, the previously measured thermodynamic data for the CROP of EtOx in acetonitrile, by Wiesbrok *et al.*,[46] correspond well to the data obtained for ethyl acetate.

Furthermore, since higher molecular weight PEtOx polymers are desired for further applications, regarding drug delivery with longer retention times and the application of PAOx as excipient for oral drug formulation, a preliminary test to synthesize a defined higher molecular weight PEtOx polymer was performed in ethyl aceate. Thereby a PEtOx with target molecular weight of 70,000 g/mol is made, at 60 °C. The synthesis is performed in accordance to a recently filed patent application, which describes the possibility to synthesize defined high molecular weight PAOx in the presence of chlorobenzene as polymerization solvent.[47] The synthesis involves the use of the sacrificial initiator method (SIM, more details *vide* **chapter 4**). Thereby, the polymerization mixture, consisting of EtOx and ethyl acetate, is purified over a crystalline 2-

phenyl-2-oxazolinium tetrafluoroborate salt. After the purification, the mixture is distilled into a polymerization flask containing the amount of initiator needed for the reaction. Finally, after 7 days' reaction time (at 60 °C) the PEtOx polymer was obtained.

In **Figure 9B** the size-exclusion chromatography (SEC) results are shown against PEtOx standards, revealing an M_n of 78,000 g/mol and Đ of 1.26 despite the presence of some low molecular weight tailing and the appearance of a double molecular weight shoulder. This first test reveals the feasibility of ethyl acetate for the synthesis of high MW synthesis of PEtOx, albeit there is still some room for further optimization (see **chapter 4, page 135**; herein the synthesis of a 25,000 g/mol PEtOx polymer in ethyl acetate on 200 g scale in triplicate is described).

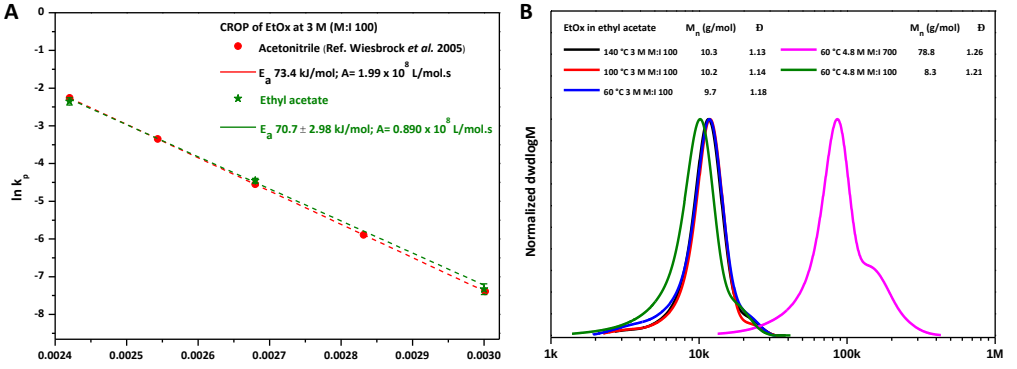


Figure 9. A) Arrhenius plot showing the linear correlation between the ln of the propagation rate constant versus the inverse temperature for the CROP of EtOx in acetonitrile (red) and ethyl acetate (green). **B)** Size-exclusion chromatography (SEC) plot for the CROP of EtOx in ethyl acetate for PEtOx polymers with M:I 100 and M:I 700. SEC was performed in *N,N*-dimethyl acetamide containing LiCl and all data samples were compared against PEtOx standards.

Another, last important concern about the green synthesis method rises when looking towards the work-up conditions of the reaction. State-of-the-art polymer synthesis of PAOx nowadays involves evaporation of the polymerization solvent, followed by re-dissolving the residue in dichloromethane and precipitation in cold diethyl ether Furthermore, if a degree of conversion below 100 % is targeted, monomer residues are still present, which are harmful and toxic.

In contradiction to previously reported work-up methods, the work-up performed of the PEtOx synthesized in ethyl acetate only involved the co-evaporation with water, followed by dialysis and freeze drying. In **Figure 10,** the ^{1}H NMR spectrum of the purified 78,000 g/mol PEtOx polymer is shown revealing the absence of traces of ethyl acetate. Although those improvements to the work-up procedure could in principle also be performed after the CROP of EtOx in *e.g.* acetonitrile, it is experimentally observed that ethyl acetate is more easily removed.

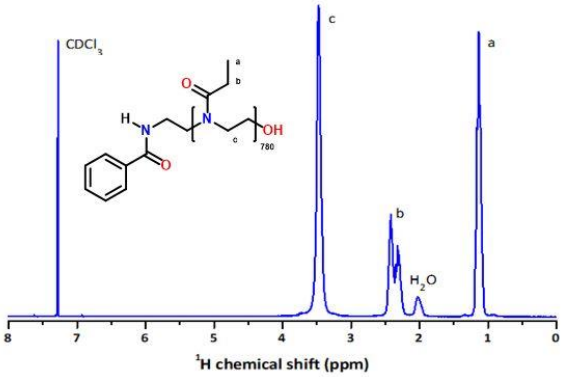


Figure 10. Proton NMR (300 MHz, Bruker) in deuterated chloroform of the purified poly(2-ethyl-2-oxazoline) polymer with a molecular weight of 78,000 g/mol. (CH2CH2 backbone, 3.5ppm (s); CH2 side-chain, 2.4 ppm (d) and CH3 side-chain, 1.2 ppm (s))

3.4 CONCLUSIONS AND OUTLOOK

In this chapter an overview is provided for the CROP of EtOx in different aromatic, highly polar and green solvents. It is remarkable that both high temperature and low temperature regimes, *i.e.* 140 °C and 60 °C respectively, show different trends in polymerization rates.

In general, at high temperatures one can say that high boiling, highly polar solvents such as sulfolane and nitrobenzene can increase the propagation rate by two-fold, compared to the standard polymerization solvent, acetonitrile. Although it is found that the double MW shoulder is more present in more polar solvents, these solvents are suitable for a fast and straightforward synthesis PAOx polymers with an M_n below 20,000 g/mol for which chain transfer reactions are less detrimental. An exception to this observation, which cannot be directly explained, is the results found for DMPU with only a 1.3-fold acceleration of k_p compared to acetonitrile.

Otherwise, at 60 °C it is shown that the non-polar aromatic solvent chlorobenzene accelerates the CROP up to 1.5 fold. From the other solvents which were screened, only sulfolane shows the same increased k_p, which could be attributed to its high polarity. Since, more defined polymers are obtained in apolar solvents, high MW PEtOx should preferably be synthesized in chlorobenzene.

The k_p´s at the different temperatures do best correlate with the boiling points of the different polymerization solvents, with exception for the CROP of EtOx in chlorobenzene.

A final interest raised from the possible polymerization in ethyl acetate. In the mindset of using PAOx for biomedical applications, the synthesis is preferably performed in a non-toxic and green solvent. Therefore, in the last part of the chapter we discuss the polymerization kinetics and reproducibility of the CROP of EtOx in ethyl acetate. In addition to the other kinetic studies in the previous sections, we compared the data of three different stock solution at all three different temperatures, for reproducibility. Finally, to check the possibility of synthesizing high MW PEtOx in ethyl acetate as polymerization solvent, a rather well-defined 78,000 g/mol PEtOx polymer (Đ 1.26) has been prepared and characterized successfully.

In future research, the CROP of EtOx could be investigated in even more solvents, *e.g.* by screening more aromatic solvents with different substituents such as dichloro and trichlorobenzene as well as bromobenzene compared to the ones that have been investigated. In addition, although it is difficult to find an alternative solvent with the same properties of sulfolane, hexamethylphosphoric triamide (HMPT) is reported to be one of the most polar solvents that exist. Despite HMPT´s high toxicity and carcinogenic properties, it could be examined as solvent for the CROP of 2-oxazolines to confirm the observed trends for sulfolane. Finally, theoretic modelling of the CROP of EtOx in the different solvents could be applied to gain more insights into possible mechanistic explanations of the observed trends.

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Chapter 4

CHAPTER 4

4 LARGE SCALE SYNTHESIS OF POLY(2-OXAZOLINE)S

ABSTRACT Upscaling a polymerization reaction asks tremendous efforts beyond the generally known issues, such as heat-transfer and mass transfer. First of all, it is important to work with optimized reagents, including solvent and initiator system, and to ensure that these are available at large enough scale. From **chapters 2 and 3**, it could be concluded that for the synthesis of high molar mass poly(2-oxazoline)s, it is best to work with apolar solvents, such as chlorobenzene or ethyl acetate, and at low temperatures, to obtain a defined PAOx polymer. Secondly, the importance of the use of a right initiator system, *i.e.* a preformed oxazolinium salt, will be elaborated throughout the introduction of this chapter. Preliminary data of Monnery *et al.* showed the use of such initiator system for the preparation of defined high molar mass PAOx, to be successful. Nevertheless, all of these earlier experiments were performed on small scale (1 g), and the optimal conditions need to be confirmed at large scale. Therefore, in this chapter an overview is given on all the efforts (and failures) being done to increase the scale of the synthesis of defined high molecular weight PAOx *via* the sacrificial initiator method. It is hereby crucial to achieve good batch-to-batch reproducibility, high purity of the final polymers and simplified procedures for the larger scale production of defined high molar mass PAOx polymers. This, in order to make the synthesis valuable for larger scale pharmaceutical applications, such as oral drug formulation. Especially for approval of the regulatory agencies the repeatability of the synthesis is highly important.

Contributors to this work

Dr. Bryn D Monnery: Pioneer that performed the synthesis of the high molar mass poly(2-oxazoline)s on small scale and involved in conceptual discussion.

Ali Tigrine: as part of his master thesis under my guidance he was involved with the start of the upscaling experiments (lab work) and continued working for Ultroxa® for the production of large scale high molar mass poly(2-oxazoline)s of the standards together with me.

Contribution of the candidate

Involved in the optimization, synthesis, characterization and data analysis of all large scale reactions of high molar mass poly(2-oxazoline)s.

Chapter 4

4.1 SACRIFICIAL INITIATOR METHOD: INTRODUCTION

Poly(2-oxazoline)s are highly interesting for biomedical applications, as repeatedly stated in literature and in the previous chapters.[1–4] Nevertheless, it is relatively hard to deal with their synthesis. The cationic ring-opening polymerization (CROP) of 2-oxazolines encounters similar challenges as anionic polymerization techniques, requiring high purity of reagents and solvents, and moisture free conditions. It was even believed to be impossible to synthesize defined PAOx polymers exceeding a molecular weight (MW) of 20,000 g/mol.[5,6] Only two years ago a patent publication by Monnery *et al.* described the synthesis of defined high MW PAOx polymers.[7] Thereby it is of utmost importance to work with extremely pure reagents, high vacuum schlenk line techniques and low temperature polymerization; to limit extrinsic and intrinsic side reactions, such as chain-transfer by β-elimination.[8] The method proposed for the synthesis of those high MW PAOx polymers, is the so-called sacrificial intiator method (SIM) closely resembling anionic polymerization techniques.[9,10] This SIM comprises the stringent purification of reagents, *i.e.* solvent and monomer, over a ´sacrificial´ amount of initiator, being an oxazolinium salt.[6,11,12] The idea is that the final residual impurities react towards PAOx oligomers, followed by a static distillation of the remaining ultrapure monomer and solvent into the final polymerization flask, containing a second amount of oxazolinium initiator needed for the polymerization.

Preliminary data (**Figure 1**), from Monnery *et al.* as published in the patent,[7] show size-exclusion chromatography (SEC) results for the synthesis of defined high MW PEtOx standards with a MW up to 110,000 g/mol prepared on small scale (1-10 g scale) at a low temperature of 42°C in the non-polar, rate accelerating, polymerization solvent chlorobenzene. Apart from these data no literature publication has ever reported the synthesis of high MW PAOx, so far.

The ability to synthesize high MW PAOx polymers opens up possibilities to use them in *e.g.* PAOx-drug conjugates, which need a long circulation time in the blood after administration.[1,13–17] Also for the use of PAOx as matrix excipient for oral solid dosage formulations - the goal of this thesis - high MW polymers are preferred.[18–22] Within this application, it is important to reduce or avoid absorption and improve the processability and the mechanical properties of the polymer, by making the polymer ´long enough´, while drug delivery is either enhanced or sustained, depending on the nature of the drug (hydrophilic vs hydrophobic). This will be completely elaborated in **chapter 5 and 6**; and is only briefly mentioned here to stress the importance of the work described in this chapter.

Additionally, it is highly desired, taking into account all these interesting application possibilities, to scale up (initially towards ± 200g scale[i]) the synthesis of these high MW PAOx polymers, in a reproducible manner. Even though, this upscaling may sound straightforward, a lot of challenges, optimization and insights in the polymerization mechanism of 2-oxazolines is needed to achieve this. Of course many examples can be found in literature, on the difficulties that are encountered during upscaling. An overview of the efforts that have been made during this thesis, to develop large scale synthesis of defined high MW alkyl-PAOx polymers can be found in this chapter including the state-of-the- polymerization, large scale synthesis steps for monomer and initiator towards high MW PAOx polymers, synthesis of the standards and the variation on sampling a big scale reaction to probe the homogeneity.

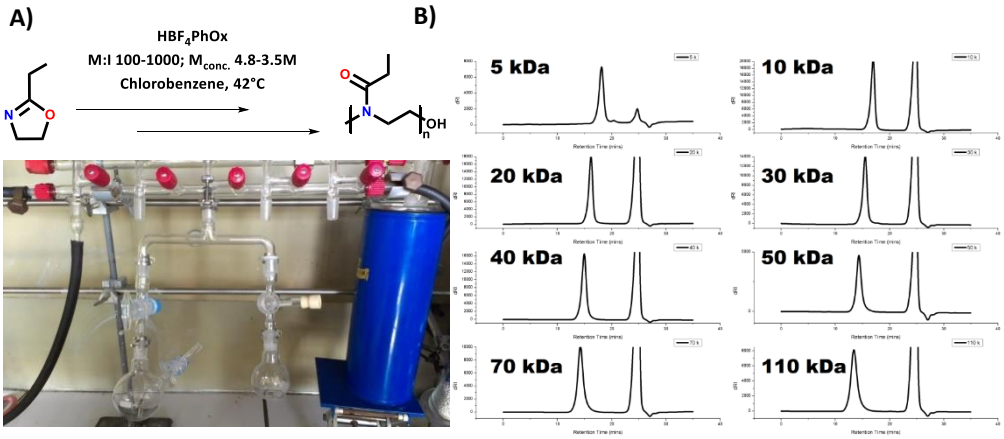

Figure 1. A) An example of a typical set-up for the sacrificial initiator method consisting of a two-manifold schlenk line, including vacuum and high purity argon. A static distillation bridge with two round bottom flasks and three-way taps. **B)** The results of a series of poly(2-ethyl-2-oxazoline) polymers with varying molecular weight of 5,000-110,000 g/mol, and dispersity, Đ, of less than 1.1. From ref.[7]

One of the biggest challenges in my project, besides the optimization of the CROP of 2-oxazolines that can be found in chapter 2 and 3, was to ´produce´ (reproducible[ii]) large batches of defined high MW PEtOx and poly(2-*n*-propyl-2-oxazoline) polymers (and at a later stage also PMeOx polymers) to be used as excipients for oral drug formulations. Preferably a series of different MW should have been synthesized. This challenge of increasing the production scale can be divided in some sub-challenges, including in a first step upscaling of monomer

[i] Because of limitation reaction vessel with regard to uniform heating and stirring capacity of normal stir bars, supposed to be easily translatable towards 1 kg scale with the help of mechanical stirring and homogenous heating.

[ii] which is desirable for the regulatory agencies (FDA, EMA) and use of these polymers in pharmaceutical applications

synthesis and solvent purification methods (**section 4.3.1**), secondly upscaling of the initiator synthesis (**section 4.3.2**) and finally applying the SIM on a larger scale (**section 4.3.3 and 4.3.4**).

4.2 MATERIALS AND METHODS

4.2.1 Materials and equipment

Materials

EtOx was kindly donated by polymer chemistry innovations (PCI). BaO, HBF_4 aqueous solution, H_2SO_4, $NaHCO_3$, $MgSO_4$, MeOH, 2-phenyl-2-oxazoline, butyronitrile, ethanolamine, zincacetate and chloromethyl(trimethyl)silane (TMS-Cl) are bought from Sigma Aldrich. Chlorobenzene and ethyl acetate (acroseal® grade) were purchased from thermofisher scientific. Diethylether, dichloromethane, petroleum ether and acetone were obtained from Merck.

Purification methods

EtOx was distilled over barium oxide under inert argon atmosphere before its use in the polymerizations. For the **purification of chlorobenzene**, first a washing step with sulfuric acid is performed. To 500 mL chlorobenzene, 60 mL sulfuric acid is added. The mixture is stirred at 850 rpm for 30 min. Afterwards an extraction is performed, with on top the chlorobenzene and at the bottom the sulfuric acid phase. This procedure is repeated until the sulfuric acid phase remains colorless, which is mostly the case after three washing steps. The chlorobenzene is then washed three times with 50 mL of an aqueous solution of sodium bicarbonate (13.05 g in 150 mL water). Between each washing step, the solution is stirred for 30 min at 1000 rpm. During extraction, the chlorobenzene is at the bottom and the aqueous phase is on top. During the first washing step some heavy bubbling is observed, due to the formation of carbon dioxide. Finally, the chlorobenzene is washed three times with (distilled) water. Between each step the mixture is again stirred for 30 min at 1000 rpm. After these washing steps, the chlorobenzene is dried over magnesium sulphate, which is followed by an additional drying step over barium oxide is performed. This solution is stirred overnight at 60 °C at 850 rpm. The final distillation to dryness is performed under argon overpressure. **Note:** As final distillation step EtOx, *n*PrOx, chlorobenzene and ethyl acetate were purified over living PEtOx polymer prior to SIM.

Equipment

Conversions of the CROP of the selected 2-oxazoline monomers were monitored by gas chromatography (GC) analysis. GC was performed on an Agilent 7890A system equipped with a VWR Carrier-160 hydrogen generator and an Agilent HP-5 column of 30 m length and 0.320 mm diameter. An FID detector was used and the inlet was set to 240 °C with a split injection ratio of 25:1. Hydrogen was used as carrier gas at a flow rate of 2 mL/min. The oven temperature was increased with 20 °C/min from 50 °C to 120 °C, followed by a ramp of 50 °C/min. to 240 °C.

All SIM solutions (and samples) were prepared in a VIGOR Sci-Lab SG 1200/750 Glovebox System with a water concentration ≤ 0.1 ppm.

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50 °C equipped with two PLgel 5 µm mixed-D columns in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent is *N,N*-dimethylacetamide (DMA) containing 50 mM of lithium chloride at an optimized flow rate of 0.5 mL/min. The spectra were analyzed using the Agilent ChemStation software with the GPC add on. Molar mass and dispersity (Đ) values were calculated against polymethylmethacrylate standards from PSS and in-house PEtOx standards, with confirmed mass from SEC with multi-angle light scattering (MALS) detector.

Light scattering (LS) measurements are performed on a 3-angle static light scattering (MALS) detector, *i.e.* miniDAWN TREOS, from Wyatt Technology. The detector is coupled on-line to an Agilent 1260 infitiny HPLC system (*vide* DMA-SEC), and used to determine absolute molar mass of the analyzed polymer samples. The measurements are performed at ambient temperature, i.e. no temperature control unit is supplied/installed with the above mentioned LS detector. The refractive index (RI) increment (dn/dc) values (*vide* experimental details) are either used as reported for the certain polymer in DMA or determined *via* online size-exclusion chromatography (SEC) equipped with an RI detector, which measures the RI increase for a 1-10 mg/mL concentration series of the mentioned polymers. The LS results are further analyzed with the provided Astra 7 software, also designed by Wyatt Technology.

Proton nuclear magnetic resonance (1H NMR) spectra were recorded on a Bruker Avance 300 MHz spectrometer, at room temperature. The chemical shifts are given relative to trimethylsilane (TMS).

Liquid chromatography coupled to electrospray ionization-mass spectrometry (LC ESI-MS) spectra were acquired on a quadrupole ion trap LC mass spectrometer (Thermo Finnigan MAT LCQ mass spectrometer) equipped with electrospray ionization (ESI).

4.2.2 Experimental methods

Monomer purification

Distillation of EtOx is performed with an extra-large set-up, starting from a 3 L round bottom flask. After each monomer purification a test polymerization for the preparation of a PEtOx DP100 polymer that should have a Đ below 1.2 is performed to ensure the purity of every distilled batch of monomer.

Synthesis of 2-phenyl-2-oxazolinium tetrafluoroborate salt HPhOx-BF_4*: initiator*

The initiator synthesis consists of two main steps including the purification of the 2-phenyl-2-oxazoline (PhOx) followed by the precipitation of PhOx in tetrafluoroboric acid (HBF_4). First a vacuum distillation of PhOx (preferably high vacuum $<10^{-1}$-10^{-2} mbar) is performed (Boiling point: 94 °C at 10^{-2} mbar; DrySyn temperature: 120 °C). The first fraction, 5 mL, was discarded when the temperature was stable. Then, the 2-way receiving joint was switched to the final receiving flask (Note: the flask should not be distilled to dryness). The resulting colorless PhOx is kept in the fridge after distillation. For the following step, 100 mL of purified PhOx is added dropwise to a solution of 100 mL HBF_4 and 100 mL methanol that is cooled to 0 °C in a 500 mL Erlenmeyer, while stirring, *via* an addition funnel. After addition, the solution is brought up to room temperature. For the crystallization of the initiator salt, the solution is put in the freezer at -20 °C overnight. Filtration was also performed in the freezer, since the crystals very easily re-dissolve when the solution warms up. Repeated recrystallization of the HPhOx-BF_4 in methanol is necessary until a white and non-smelly product is obtained. The final white product, HPhOx-BF4 product, has been stored in a vacuum oven at 50 °C until use. Characterization is performed by proton NMR and LC ESI-MS revealing more than 99 % purity. Proton NMR chemical shifts: 12.2 ppm, oxazolinium proton; 8.5-7.5 ppm, aromatic protons; 5.2 ppm, proton next to O and 4.3 ppm, proton next to N of the oxazoline ring.

Synthesis of 2-n-propyl-2-oxazoline

Butyronitrile (384,72 ml; 4.42 mol) and ethanolamine (293,45 ml; 4,86 mol) were mixed together, and zincacetate (19,40 g; 0,0884 mol) was added in a catalytic amount.[6,23–25] The mixture was heated to 130 °C for 3 days while stirring at 850 rpm. A Vigreux column was put on

top of the round bottom flask to permit the mixture to reflux. During the reaction the system was flushed with argon to permit the formed ammonia to escape. Ammonia will act as a nucleophile during the polymerization and it is highly important to remove all the ammonia. Over time a color change of the solution was observed, going from colorless to yellow to red and finally to black. This is due to the oxidation of ethanolamine.

Directly after the reaction, the residual mixture was fractionally distilled at 75 °C and 10^{-1} mbar. The first 20 mL were discarded and the distillation was not performed until dryness. The nPropOx was dried by adding BaO and ninhydrin (both as indicator for ammonia and to react away the residual ammonia). Further purification proceeds by adding sodium and heating the mixture for 2 h at 120 °C (molten sodium purification). A last purification step was done by adding $HPhOxBF_4$ salt to polymerize a small fraction of the monomer followed by distillation.

Synthesis of 50g scale poly(2-oxazoline) standards at low temperature of 42 °C and synthesis of 200g scale poly(2-oxazoline)s at 60 °C.

Both the 50 g scale synthesis of the PEtOx standards and the 200 g scale synthesis of the defined high MW PEtOx and P*n*PrOx polymers, are made according to the sacrificial initiator method (SIM). In **Figure 2**, the set-up for the 50 scale synthesis is shown, including the glassware for the static distillation (**Figure 2, n° 5**) which can be connected to the schlenk line and taps (**Figure 2, n° 3 and 4**) to isolate either the ´cleaning´ (**Figure 2, n° 2**) or polymerization flask (**Figure 2, n° 1**). Every flask is foreseen of a rare-earth magnetic stir bar (**Figure 2, n° 6**). A similar, but larger, set-up was used for the 200 g scale polymerizations.

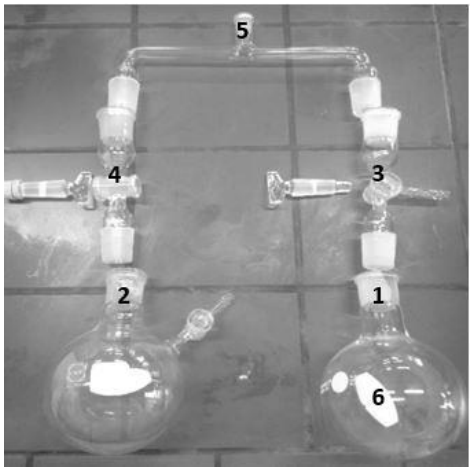

Figure 2 Experimental set-up that was used for the synthesis of the PEtOx standards at 50g scale.

In the following, the different steps for the CROP of EtOx *via* the SIM are described.

Step 1: Silanization of the reaction set-up to remove water attached to the glassware

First the set-up, which is connected to the Schlenk line, is silanized with TMS-Cl. The TMS-Cl is spread over the set-up by performing one freeze-pump-thaw cycle[iii]. After the silanization procedure the set-up is dismantled from the Schlenk line and cleaned with water, acetone and petroleum ether.

Step 2: Addition and melting of the initiator (HPhOx-BF_4)

Before the ´cleaning´ and polymerization flasks are connected to the Schlenk line, they are filled with the required amount of initiator. Generally, the amount of initiator used to purify the monomer and solvent mixture in the cleaning flask is 1 mol% of the monomer concentration. The initiator is molten under vacuum and left for drying overnight.

Step 3: Addition of the monomer and solvent to the cleaning flask

The monomer and solvent are volumetrically added in a glove box. Then, the flask is connected to the set-up on the Schlenk line. Afterwards the mixture is being polymerized to purify the monomer and solvent for 30 min at 60 °C.

Step 4: Freeze-pump-thaw cycles monomer and solvent (under high vacuum)

The mixture in the cleaning flask is after the ´cleaning/purifying´ in step 3, cooled down with liquid nitrogen. Next, the completely frozen mixture is subjected to high vacuum (< 10^{-3} mbar), for 15 min, while kept frozen. After closing the vacuum connection to the schlenk line, the mixture is thawed completely and undergoes a degassing step. This freeze-pump-thaw cycle is repeated at least three times, while kept under vacuum. The mixture is degassed until the vacuum does not go above 10^{-2}-10^{-3} mbar anymore, *i.e.* the vacuum is kept stable for the static distillation.

Step 5: Static distillation monomer and solvent to the polymerization flask

While kept under vacuum, the cleaning flask is thawed for the last time. Simultaneously, the polymerization flask is cooled down with liquid nitrogen. Due to the reversed temperature gradient, a static distillation is carried out whereby the monomer and solvent from the cleaning flask are distilled into the polymerization flask. The distillation is completed when the cleaning

[iii] Freeze-pump-thaw cycle includes freezing with liquid nitrogen, applying vacuum while frozen, close vacuum and thaw the solution.

flask only contains residues of oligomer impurities. Note: for the 200 g scale, an oil bath is used for heating the cleaning flask to 40 °C to 60 °C to accelerate the static distillation. Additional heating tape is applied on the distillation bridge, with a temperature set at 30 °C to 50 °C, generally 10 °C lower than the temperature set of the oil bath to ensure that the monomer and solvent remain in the gaseous phase.

Step 6: Start of the polymerization

The system is brought under argon after the static distillation. The polymerization flask is then disconnected from the schlenk line set-up and put in an oil bath at 60 °C, for the time needed for the polymerization.

An overview of all the amounts of initiator, monomer and solvents that were used for the large scale polymerizations of PEtOx and P*n*PrOx polymers as described in **section 4.3**, can be found in **Table 1** together with their theoretical MW, reaction times and reaction scale.

Table 1. Overview of all the polymerization reactions which are performed at large scale including the target length of the polymer, concentration of monomer, amount of initiator, solvent and monomer, and polymerization times. (DP = degree of polymerization, PhCl = chlorobenzene and EtOAc = ethyl acetate)

Polymer type	MW (theoret.) (DP / kDa)	Initiator (g)	Solvent (type + mL)	Monomer (conc./ mL)	Reaction time (days)	Scale (g)
PEtOx_50kDa_1	500 / 50	1.003	PhCl/ 322.5	4.8/ 305.1	5	200
PEtOx_50kDa_2	500 / 50	1.003	PhCl/ 322.5	4.8/ 305.1	5	200
PEtOx_50kDa_3	500 / 50	1.003	PhCl/ 322.5	4.8/ 305.1	5	200
PEtOx_100kDa_1	1000 / 100	0.426	PhCl/ 472.7	3.5/ 259.2	12	200
PEtOx_100kDa_2	1000 / 100	0.426	PhCl/ 472.7	3.5/ 259.2	12	200
PEtOx_100kDa_3	1000 / 100	0.426	PhCl/ 472.7	3.5/ 259.2	12	200
PnPrOx_50kDa_1	443/ 50	1.006	PhCl/ 238.6	4.8/ 312.5	5.5	200
PnPrOx_50kDa_2	443/ 50	1.006	PhCl/ 238.6	4.8/ 312.5	5.5	200
PnPrOx_50kDa_3	443/ 50	1.006	PhCl/ 238.6	4.8/ 312.5	5.5	200
PEtOx_25kDa_1	250 / 25	1.903	EtOAc/ 301	4.8/ 285.7	2-3	200
PEtOx_25kDa_2	250 / 25	1.903	EtOAc/ 301	4.8/ 285.7	2-3	200
PEtOx_25kDa_3	250 / 25	1.903	EtOAc/ 301	4.8/ 285.7	2-3	200
PEtOx_12kDa_50g	100/ 10	1.169	PhCl/74.56	4.8/ 71.4	4	50
PEtOx_23kDa_50g	200/ 20	0.587	PhCl/75.142	4.8/ 71.4	5.5	50
PEtOx_44kDa_50g	400/ 40	0.293	PhCl/75.44	4.8/ 71.4	14.5	50

PEtOx_69kDa_50g	600/ 60	0.197	PhCl/130	4/ 71.4	28	50
PEtOx_96kDa_50g	800/ 80	0.147	PhCl/130	3.5/ 71.4	41	50
PEtOx_191kDa_50g	1600/ 160	0.0735	PhCl/130	3.5/ 71.4	81	50

Refractive index increment determination (dn/dc) via online size-exclusion chromatography for PMeOx, PEtOx and PnPrOx homopolymers

In order to able to measure the absolute molar mass of the synthesized polymers (*vide infra*) *via* SEC-MALS, the refractive index increment (*i.e.* the dn/dc value; **Figure 3**) of these particular polymers has to be determined. Therefore, six different concentrations of a polymer standard, with a particular MW and chemical composition, were measured on SEC. Preferably the molar mass of this standard sample is known and is situated in the middle range of the absolute MW determination. Nonetheless, for homopolymers the dn/dc holds almost for the whole range of MW and is almost unaffected as a function of molar mass. In **Figure 3** an overview is given of the determined dn/dc values for the series of PAOx homopolymers, PMeOx, PEtOx and P*n*PrOx. One can see that the dn/dc values increase with the increment in polarity of the PAOx polymers respectively.

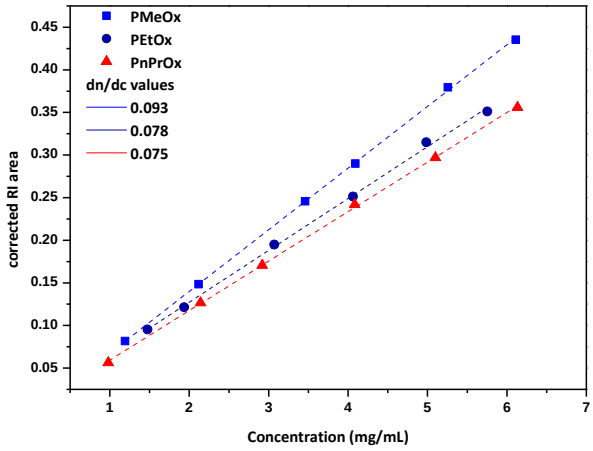

Figure 3. Overview of the refractive index increment as a function of concentration for a DP100 PMEOx, PEtOx and P*n*PrOx homopolymers with Đ below 1.1, determined *via* SEC in *N,N*-dimethyl acetamide.

4.3 RESULTS AND DISCUSSION

4.3.1 Upscaling monomer synthesis and solvent purification

Since the EtOx monomer is commercially available (by PCI), only the *n*PrOx monomer needed to be prepared and purified on large scale. Also the solvent purification has been perfomed on large scale.

Synthesis of 2-n-propyl-2-oxazoline

The *n*PropOx was synthesized on a large scale (400 mL) *via* the Witte Seeliger method, since the corresponding nitrile is commercially available. The synthesis is performed according to a described literature procedure by Hoogenboom *et al.*[26] (for a detailed description *vide* experimental, **section 4.2.2**). The exact mechanism for this reaction is not reported, but it most likely proceeds *via* an amidine intermediate (**Figure 4**).

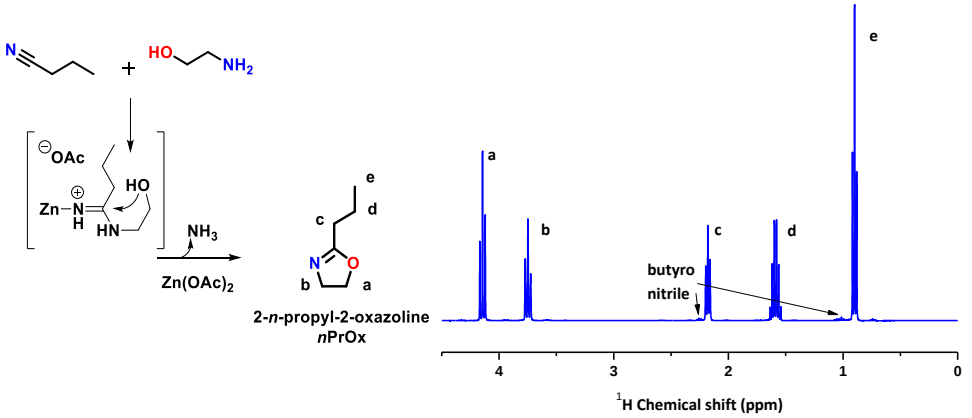

Figure 4. The scheme (**left**) shows the Witte-Seeliger reaction starting from butyronitrile towards 2-*n*-propyl-2-oxazoline including the expected amidine intermediate. Also, the proton NMR spectrum of *n*PrOx is shown (**right**), after final purification by distillation.

When performing the reaction on large scale, it was found to be difficult to get rid of the residual ammonia. Because of the nucleophilic character of ammonia, even the presence of low concentrations will induce termination during the CROP of *n*PrOx. Certainly, when aiming for high MW synthesis of P*n*PrOx this will negatively influence the reproducibility of the results. Therefore, stringent purification is performed including at minimum three distillations. A combination of a distillation over molten sodium, followed by a distillation over BaO and ninhydrin, was experimentally found to be the most effective. The purity of *n*PrOx was confirmed

by proton NMR spectroscopy (**Figure 4**). Trace amounts of butyronitrile (± 5 %) were still present, but these do not interfere with the CROP and will act as additional solvent.

4.3.2 Large scale initiator synthesis

The initiator synthesis on a 100 g scale (**Figure 5**) is fairly easy and straightforward. The basic PhOx (**2**) monomer acts as a base and captures the proton from the tetrafluoroboric acid (**1**). The difficult part in the synthesis of the initiator is the purification. This has also been observed with other oxazolinium salts, which are generally difficult to isolate. The initiator has to be recrystallized multiple times from methanol, and the precipitate is only stable at -20 °C or lower as above this temperature the crystals redissolve. It is necessary to get this initiator extremely pure, since this is the starting point of the SIM. Experimentally we developed a purification procedure in which at least three recrystallizations were involved. The product was determined to be pure when no visual coloration and nor the smell of PhOx was observed yielding the white product.

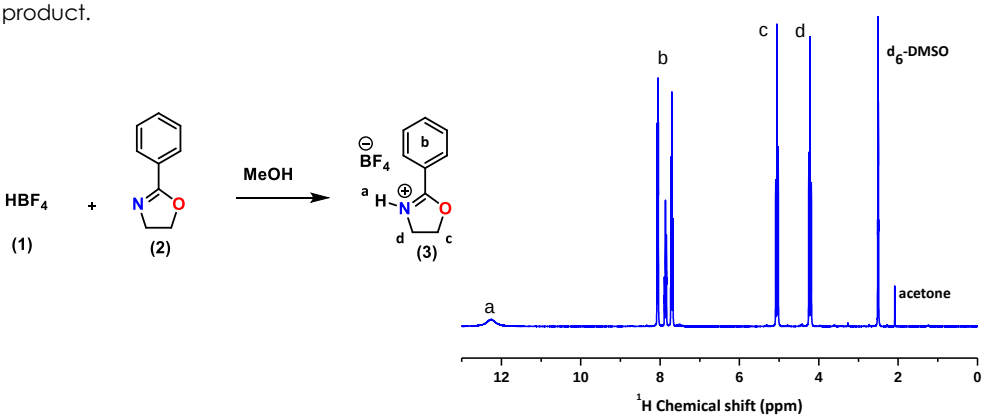

Figure 5. Synthesis scheme for the preparation of the PhOx tetrafluoroborate oxazolinium (**left**); Proton NMR spectrum of the initiator in deuterated dimethyl sulfoxide (d_6-DMSO) (**right**). (chemical shifts: 12.2 ppm, oxazolinium proton (a); 8.5-7.5 ppm, aromatic protons (b); 5.2 ppm, proton next to O (c) and 4.3 ppm, proton next to N of the oxazoline ring (d).)

4.3.3 Triplicated synthesis of high molecular weight poly(2-ethyl-2-oxazoline)s *via* SIM

As stated earlier in the introduction of this chapter, the synthesis *via* the SIM was originally developed on a small scale of approximately 1-10 g per batch (at 42 °C). Within this project to develop PAOx as polymer platform for oral drug formulations, larger amounts of polymer are needed. This upscaling process included much failure and the described results (*vide infra*) are a summary of all efforts that brought the production process successfully to a

scale where 200 g of polymer could be synthesized per batch at 60 °C[iv]. These failures were related to impurities in monomers and solvent, poor vacuum quality, leaks in the SIM set-up during static distillation and insufficient stirring.

Every single size-exclusion chromatogram in this section resembles a polymer batch of 200 g and each polymer was at least synthesized three times to demonstrate the reproducibility of the final optimized process (*vide* pharmaceutical regulatory agencies and GMP) that is shown in **Figure 6**.

Figure 6. Picture of the large scale SIM set-up consisting of two 2 L round bottom flasks, a distillation bridge, a three-way tap to isolate the prepared polymerization mixture; all connected to a two-manifold schlenk line (**left**). Picture of the SIM cleaning step set-up consisting of a heating plate at 40-60 °C, heating tape is applied around the distillation bridge which is set at a lower temperature compared to the heating plate, and a liquid nitrogen or diethyl ether/dry ice baths to trap the liquids during static distillation (**right**).

Synthesis of 50,000 g/mol poly(2-ethyl-2-oxazoline)

The 50 kDa PEtOx polymer was the first polymer that was synthesised in triplicate on large 200 g scale (**Figure 7**). The optimal monomer concentration for a DP500 polymer was kept at 4.8 M, which was found to be the optimal concentration according to observations by Monnery *et al*.[7] To reduce the reaction time to 5 days at 60 °C, it was opted to target a higher DP and terminate the reaction at ± 70 %. Since the CROP of EtOx follows linear pseudo-first order kinetics, it is inherent that the conversion slows down over time, depicted as the ´slow plateau´ region of the degree of conversion versus time plot (**Figure 7, right**). It is important to stay in the ´initial fast´ region when aiming for a relatively fast polymerization reaction. As an example a 50,000 g/mol PEtOx polymer can be produced in ± 5 days reaction time when a DP700 is targeted and terminated at ± 70 % conversion; whereas a ± 8 days reaction time is required when a DP525 is terminated at 95 % conversion. An optimum should be found between time and conversion,

[iv] For the large 200 g scale synthesis of the high MW PAOx polymers it was opted to synthesize the polymers at 60 °C, to reduce the reaction times.

because 30 % of monomer feed is lost when aiming for 70 % conversion, which however may be recovered in an industrial set-up (**Figure 7**).

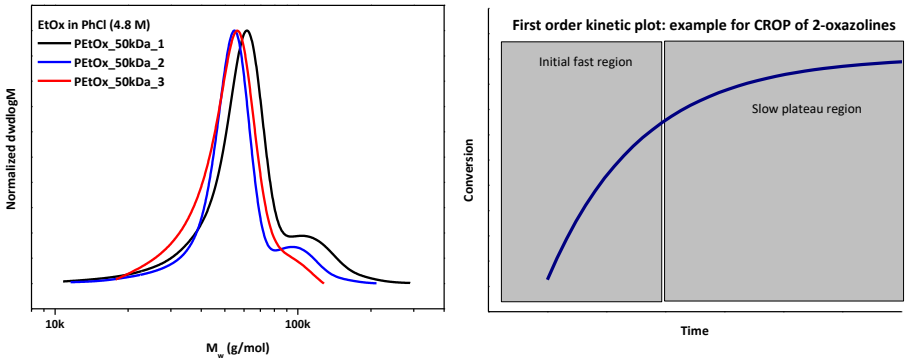

Figure 7. Triple synthesis of 50,000 g/mol poly(2-ethyl-2-oxazoline) in chlorobenzene on 200 g scale (**left**); degree of conversion versus time plot for the linear first order kinetics resembling the reaction mechanism for the CROP of EtOx. (**right**).

Table 3. Molecular weight analysis for the triple synthesis of a 50,000 g/mol PEtOx polymer by SEC analysis calculated against PEtOx standards, PMMA standards and by LS detection, respectively. (np = not purified yet)

Batch	M_n (PEtOx)	M_p (PEtOx)	Đ	M_n (PMMA)	M_p (PMMA)	Đ	M_p (LS)	Đ (LS)
	kDa	kDa		kDa	kDa		kDa	
PEtOx-50kDa-1	54	62	1.22	85	93	1.15	**55.5**	**1.1**
PEtOx-50kDa-2	51	54	1.14	84	89	1.12	**48.7**	**1.04**
PEtOx-50kDa-3	47.2	56.3	1.15	85.0	96.9	1.11	np	np
Average and standard deviation	**50.7 ± 3.4**	**57.4 ± 4.1**	**1.17 ± 0.04**	**84.7 ± 0.6**	**93.0 ± 4**	**1.13 ± 0.02**	**52.1 ± 4.8**	**1.07 ± 0.04**

Synthesis of 100,000 g/mol poly(2-ethyl-2-oxazoline)

The synthesis for the 100,000 g/mol PEtOx is similar to that of the 50,000 g/mol PEtOx polymer that is described above. The only additional difficulty that is encountered is the increased viscosity of the reaction medium, that comes along with making very high MW polymers in general. Therefore, the optimal monomer concentration was found to be 3.5 M. At this concentration, control over the molar mass and Đ is maintained, as seen in the results in **Figure 8 and Table 4**. The reaction time for this polymer is approximately 12 days, when aiming for a DP 1400 and ± 70 % conversion. It is observed that below this concentration boundary, the control over the CROP of EtOx was lost, with both low and high MW tailing in the molar mass distribution. This poorer control could be attributed to the very low initiator concentration present in the solution. It is

experimentally observed that it does not work below an initiator concentration of 2.5 mM, but not known why. One can also see that the double MW shoulder of the 100,000 g/mol PEtOx (prepared at 3.5 M) increases, compared to the SEC diagram of the 50,000 g/mol polymer (4.8 M monomer concentration). This is a general trend that is observed for the CROP of 2-oxazolines when aiming for higher MW, because the likelihood of chain transfer to monomer increases when twice the MW is targeted and, thus, more monomer is present.[5] It should be noted that for the intended goal of this project, *i.e.* the use of PAOx for drug formulation, high MW tailing does not necessary disqualify the polymer for the application. In fact, low MW tailing and irreproducible results are more harmful with regard to toxicity and adverse reactions.

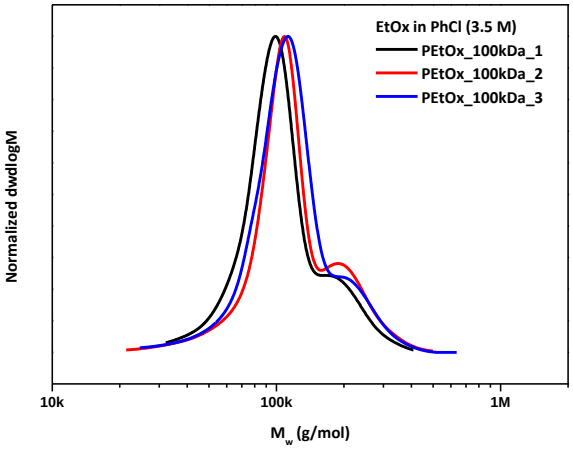

Figure 8. Size-exclusion chromatography results for the triple synthesis of 100,000 g/mol poly(2-ethyl-2-oxazoline) in chlorobenzene on 200 g scale.

Table 4. Molecular weight analysis for the triple synthesis of a 100,000 g/mol PEtOx polymer by SEC analysis calculated against PEtOx standards, PMMA standards and by LS detection, respectively.

Batch	M_n (PEtOx)	M_p (PEtOx)	Đ	M_n (PMMA)	M_p (PMMA)	Đ	M_p (LS)	Đ (LS)
	kDa	kDa		kDa	kDa		kDa	
PEtOx-100kDa-1	96	99	1.19	134	139	1.16	**98.7**	**1.1**
PEtOx-100kDa-2	105	108	1.23	167	169	1.19	**103**	**1.09**
PEtOx-100kDa-3	107	125	1.35	169	193	1.29	-	**-**
Average and standard deviation	**103 ± 6**	**111 ± 13**	**1.26 ± 0.08**	**157 ± 20**	**167 ± 27**	**1.21 ± 0.07**	**101 ± 3**	**1.10 ± 0.01**

Synthesis of 50,000 g/mol poly(2-n-propyl-2-oxazoline)

The next challenge that was addressed is the synthesis of a 50,000 g/mol P*n*PrOx. This large scale CROP concerns a non-commercially available monomer for which the purification is crucial. In **section 4.3.1**, the description regarding the upscaling of the *n*PrOx monomer synthesis can be found. Initially problems were found during the polymerization, which could most likely be attributed to the presence of trace amounts of ammonia resulting from the monomer synthesis. It was found that a harsh purification step of the *n*PrOx monomer over molten sodium is unavoidable for the production of high MW P*n*PrOx. A clear difference with the PEtOx polymers, is the lower double MW shoulder for P*n*PrOx 50,000 g/mol polymers that can be observed from the SEC diagram in **Figure 9 and Table 5**. This observation can provide insights in the β-elimination process, during the CROP of 2-oxazolines. (*vide* **chapter 1**) It is proposed in literature that chain coupling occurs less in relation to increased β-substitution of the alkyl side-chain of 2-oxazolines due to enhance steric hindrance.[6] The observation that the P*n*PrOx SEC traces reveal similar low molar mass tailing but less double MW coupling products, indicates that the occurrence of chain transfer by β-elimination is similar while coupling occurs less for *n*PrOx. The slightly lower M_n (PEtOx) values compared to the M_p (LS) for the P*n*PrOx polymers observed in **Table 5** is due to the calculation of the M_n of the synthesized P*n*PrOx polymers against the more hydrophilic PEtOx standards. The values obtained by LS correlate better with the theoretical molar mass.

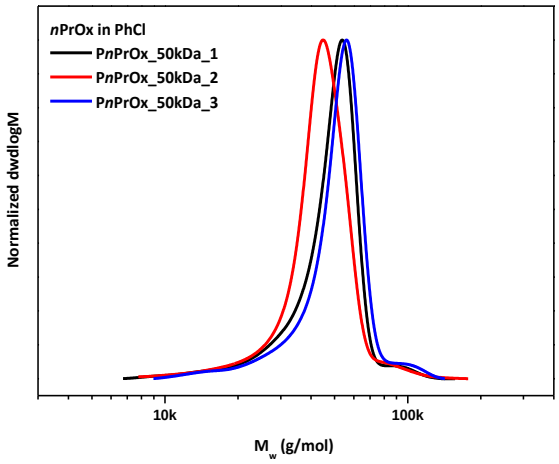

Figure 9. Size-exclusion chromatography results for the triple synthesis of 50,000 g/mol poly(2-*n*-propyl-2-oxazoline) in chlorobenzene on 200 g scale.

Table 5. Molecular weight analysis for the triple synthesis of a 50,000 g/mol PnPrOx polymer by SEC analysis calculated against PEtOx standards, PMMA standards and by LS detection, respectively.

Batch	M_n (PEtOx)	M_p (PEtOx)	Đ	M_n (PMMA)	M_p (PMMA)	Đ	M_p (LS)	Đ (LS)
	kDa	kDa		kDa	kDa		kDa	
PnPrOx-50kDa-1	42.8	53.8	1.14	66.6	80.5	1.11	**51.58**	**1.03**
PnPrOx-50kDa-2	40.0	44.9	1.13	61.3	80.2	1.18	**51.19**	**1.05**
PnPrOx-50kDa-3	45.1	56.1	1.16	69.8	83.4	1.12	**53.49**	**1.04**
Average and standard deviation	**42.6 ± 2.6**	**51.6 ± 5.9**	**1.14 ± 0.02**	**65.9 ± 4.3**	**81.4 ± 1.8**	**1.14 ± 0.04**	**52.1 ± 1.2**	**1.04 ± 0.01**

Synthesis of 25,000 g/mol poly(2-ethyl-2-oxazoline) in ethyl acetate

In **chapter 3**, it is reported that the CROP of EtOx can be conducted in the fairly ´green´ solvent ethyl acetate. A 25,000 g/mol PEtOx polymer is produced in triplicate to confirm that PEtOx can be reproducibly prepared in this solvent. SEC results are represented in **Figure 10 and Table 6** to show the reproducibility and controllability of the CROP of EtOx in ethyl acetate as promising polymerization solvent.

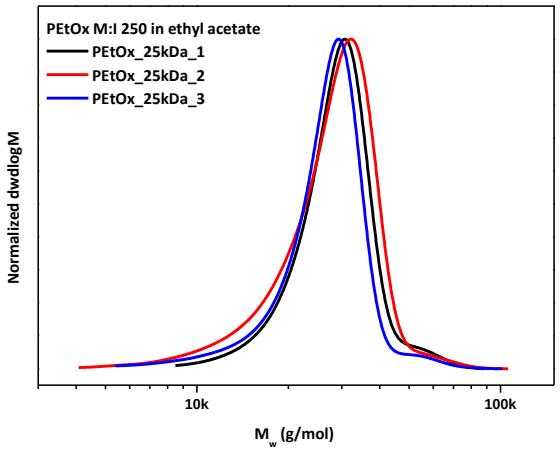

Figure 10. Size-exclusion chromatography results for the triple synthesis of 25,000 g/mol poly(2-ethyl-2-oxazoline) in ethyl acetate on 200 g scale.

Table 6. Molecular weight analysis for the triple synthesis of a 25,000 g/mol PnPrOx polymer by SEC analysis calculated against PEtOx standards, PMMA standards and by LS detection, respectively; in ethyl acetate.

Batch	M_n (PEtOx)	M_p (PEtOx)	Đ	M_n (PMMA)	M_p (PMMA)	Đ	M_p (LS)	Đ (LS)
	kDa	kDa		kDa	kDa		kDa	
PEtOx-25kDa-1	26.9	30.7	1.10	50.6	57.1	1.08	**22.7**	**1.03**
PEtOx-25kDa-2	24.6	32.2	1.17	36.3	45.9	1.13	**25.6**	**1.03**
PEtOx-25kDa-3	24.4	29.4	1.13	45.8	54.7	1.11	**24.9**	**1.06**
Average and standard deviation	**25.3 ± 1.4**	**30.8 ± 1.4**	**1.13 ± 0.04**	**44.2 ± 7.3**	**52.6 ± 5.9**	**1.11 ± 0.03**	**24.4 ± 1.5**	**1.04 ± 0.02**

4.3.4 Synthesis of PEtOx molar mass standards on large scale

Until now literature reports describe the MW characteristics of PAOx as measured *via* SEC mostly against commercial standards, such as PMMA or PEG, because it was not possible to synthesize highly defined PAOx polymers with a broad range of MW. On the contrary, we already described (*vide supra*) the MW determination of the synthesized PAOx polymers calculated against PEtOx standards, made by Monnery on a small (1 g) scale.[27] However, the synthesis of the PEtOx standards having molar masses ranging from 10,000 g/mol – 200,000 g/mol is a challenge on large scale (50 g scale). In contradiction to the PAOx polymers described in section 4.3.3, polymers that need to serve as molar mass standard need to be as defined as possible (Đ ~ 1.01-1.05). Therefore, the synthesis of the PEtOx standards on large scale is performed at a reaction temperature of 42 °C (analogous to the literature and patent by Monnery *et al.*).[6,7] Only for the synthesis of the 160,000 g/mol PEtOx standard, a temperature of 60 °C was utilized to reduce the extremely long reaction time. In general, it was observed that lower polymerization temperatures yield more defined PAOx polymers, *i.e.* due to a decrease in the amount of side reactions.[6,7] **Figure 11** shows the comparison between the SEC traces of the PEtOx standards made on 1 g versus 50 g scale. PEtOx standards below 100,000 g/mol show a ± 10 % higher Đ and double MW shoulder (in the SEC traces), if the PEtOx_50 g standards are compared to the PEtOx_1 g standards. This can probably be explained by the fact that stirring on large scale is less efficient than on small scale, inducing less homogenous reaction mixture conditions, which could give higher conversion[v] locally. Proof for this explanation is provided in the next **section**

[v] At higher conversions chain coupling becomes more significant due to higher relative concentration of less reactive enamine compared to the 2-oxazoline monomer, which induces relatively more chain coupling.

(4.3.5) of this chapter, where a large scale reaction is sampled at different spots in the reaction mixture.

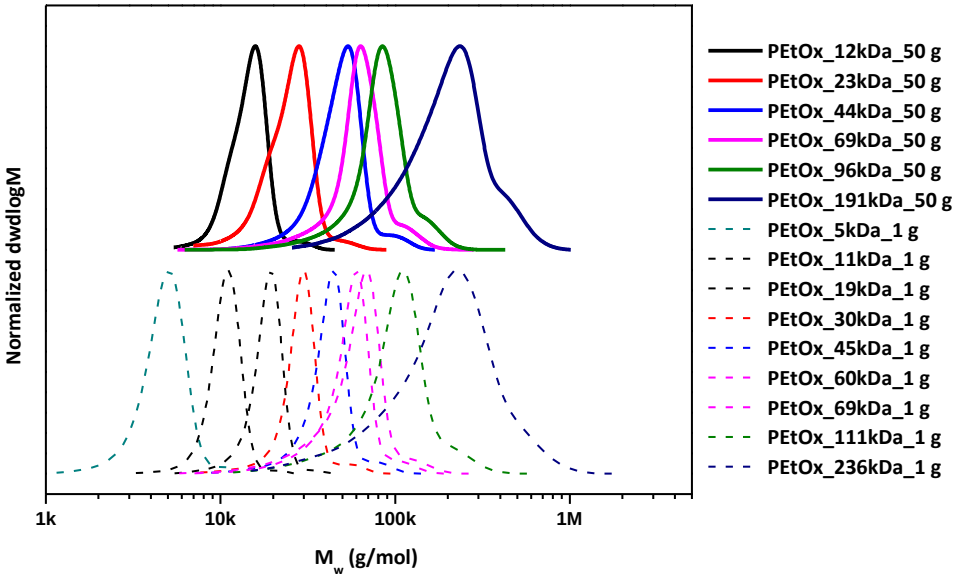

Figure 11. Size-exclusion chromatograms for the series of PEtOx standards, ranging from 10,000 g/mol to 200,000 g/mol prepared on 50 g scale (this work) and on 1 g scale as reported by Monnery *et al.*[7]

Even though, Đ values (analyzed by LS, **Table 7**) of the polymers prepared at 50 g scale (except PEtOx-std-191kDa) are situated below 1.1, efforts to use these standards as calibration for our SEC system failed. When peak molar mass values were compared (1 g versus 50 g PEtOx standards) a difference of > 5,000 g/mol could be found. Unfortunately, this is unacceptable if one wants a calibration that gives relevant and reliable results on the MW of the respective polymer. The reason for the observed broader Đ for the synthesis of the PEtOx standards at larger scale is proposed to be inhomogeneity of the large scale reactions as will be further explored in **section 4.3.5**.

To improve the Đ, it was investigated to execute fractionation experiments, to separate *e.g.* the double MW shoulder of the polymer by preparative SEC methods. Although good observations were made using preparative SEC[vi] (prepSEC), it is still only possible for small scale

[vi] Manual size-exclusion chromatography column

experiments where 1 g of polymer sample can be purified during a prepSEC column run. A feasible solution to purify these large scale PEtOx standards at once was not found up to date.

Table 7. Molecular weight analysis for the standard synthesis of a series of PEtOx polymers by SEC analysis calculated against PEtOx standards, PMMA standards and by LS detection, respectively; in chlorobenzene at 42 °C.

Batch	M_n (PEtOx)	M_p (PEtOx)	Đ	M_n (PMMA)	M_p (PMMA)	Đ	M_p (LS)	Đ (LS)
	kDa	kDa	-	kDa	kDa	-	kDa	-
PEtOx-std-12kDa	13.9	15.9	1.07	21.4	25.5	1.10	**12.32**	**1.02**
PEtOx-std-23kDa	23.0	28.3	1.11	36.3	45.7	1.13	**23.49**	**1.02**
PEtOx-std-44kDa	44.6	53.9	1.13	61.6	72.2	1.10	**43.68**	**1.05**
PEtOx-std-69kDa	56.2	63.4	1.16	86.5	97.0	1.17	**68.70**	**1.08**
PEtOx-std-96kDa	74.7	84.4	1.17	116.6	130.9	1.19	**95.74**	**1.09**
PEtOx-std-191kDa	160	237	1.40	181.6	243.4	1.24	**190.7**	**1.22**

4.3.5 Homogeneity of large scale reactions

Following the described synthesis and discussion on the results for the large scale PEtOx standard series, it is proposed that variations occur due to scaling up of these PAOx polymer reactions. Since the variation on the molar mass distribution or the Đ is more pronounced for the larger MW PEtOx standards, we started thinking if the stirring capacity on large scale is still sufficient enough. The high viscosity of the polymerization mixtures could make it difficult to homogenize the mixture, during polymerization on large scale. The question raised, if *e.g.* on the edges of the round bottom flask, the characteristics of the synthesized polymer are comparable with the ones found in close proximity of the stir bar, or not. In conclusion, we designed an experiment where during the polymerization samples were taken from three different places. In this way we try to provide proof to our above mentioned concerns of homogeneity of the polymerization mixture.

In order to understand the partial loss of control for the large scale PEtOx standard synthesis, we developed an experimental set-up to probe the homogeneity during the synthesis of a 200 g PEtOx 70,000 g/mol polymer. Everything is carried out according to the explained procedures in the experimental methods. However, during the polymerization, samples were taken at three different places in the polymerization mixtures at different time points. An example

of the set-up is shown in **Figure 12**, together with the obtained results of the MW versus degree of conversion plot for the CROP of EtOx on large scale.

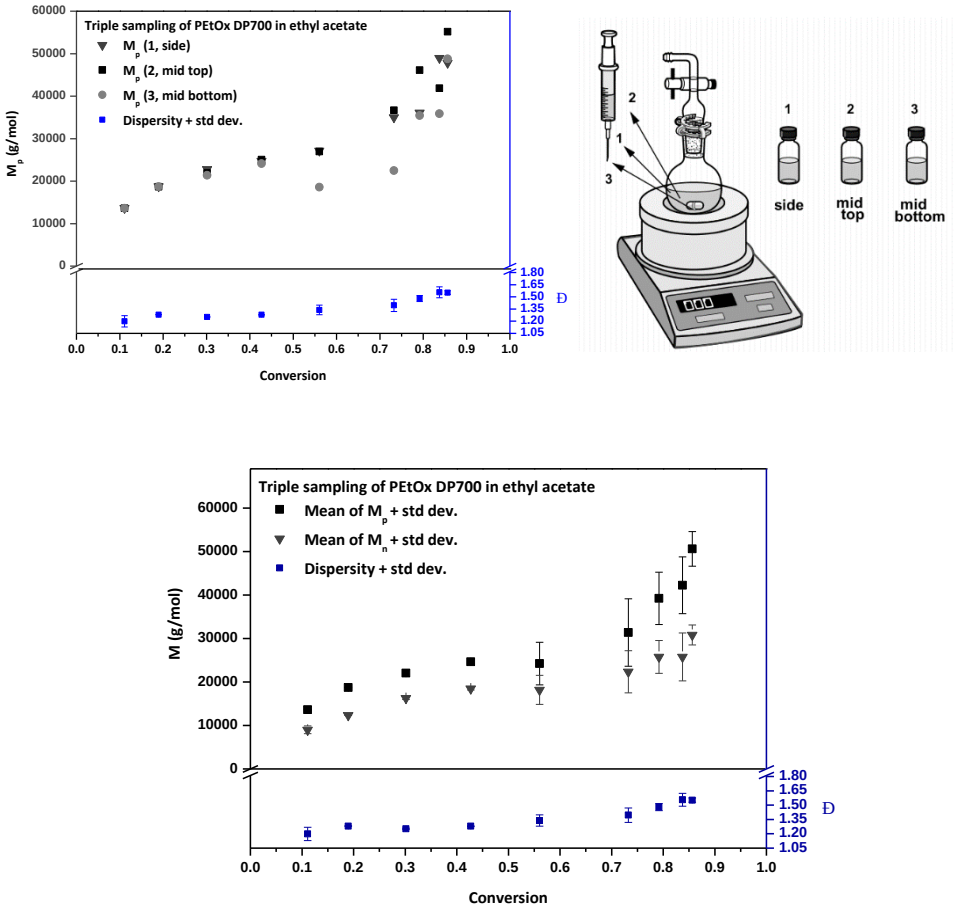

Figure 12. Molecular weight (M_n and M_p) as a function of degree of conversion plot; including error bars and Đ values. (**left top and bottom**) An example of the set-up of the polymerization of EtOx in ethyl acetate; showing the different sampling positions during the reaction. (**right, top**)

If one looks at the data obtained by sampling of the reaction at different places it is observed that until approximately 50 % conversion, no significant variation is found. From that moment on, however, the standard deviation and Đ goes up. It is remarkable that different SEC results are obtained depending on the place of sampling, which can be attributed to failure of efficient stirring during the reaction. This only becomes apparent at higher conversion where the viscosity of the polymerization mixture becomes higher. It must be noted that the increased Đ

may also (partially) be affected by the sampling procedure; causing some termination even though it is performed under inter atmosphere. It is impossible to completely cancel out the introduction of impurities while sampling. Nonetheless, this experiment shows the importance of stirring and homogenization during process upscaling. The issue that we face can be easily solved by *e.g.* mechanical stirring of the polymerization mixture which could not be developed anymore within this PhD research. In conclusion, the results from this paragraph can serve as a plausible explanation why we failed to upscale the obtained results for the synthesis of PEtOx standards at small scale; as described in the patent by Monnery *et al.*[7] This can explain also the fact that the PEtOx polymers that were synthesized at 200 g scale and higher temperature (60 °C), as reported in **chapter 3**, show slightly higher ± 10 to 15 % Đ values as a result of both inhomogeneity of the reaction mixture and higher polymerization temperatures.

4.4 CONCLUSIONS AND OUTLOOK

Since PAOx are gaining more and more interest in biomedical and pharmaceutical applications, the question was raised if it is feasible to produce defined (Đ < 1.3) PAOx (co)polymers on a larger scale. Therefore, this chapter focused on the reproducible synthesis of high MW PAOx on large scale; since this is a prerequisite to advance the PAOx polymers through the regulatory procedures. Previous work was performed at 1 g scale, which is inefficient for the targeted applications in this PhD thesis, which includes testing the PAOx platform for oral drug formulation development. One hot-melt extrusion test run already requires 5-10 g of polymer, depending on the drug content.

A lot of challenges had to be overcome, including upscaling of the (non-) commercial monomers and initiator synthesis. Additionally, results are included for the ´first´ successful upscaling tests (up to ± 200 g scale) for the CROP of 2-oxazolines. All polymerization reactions were performed in triplicate revealing good reproducibility. The PAOx polymers which were synthesized on large scale include PEtOx 25,000 g/mol, PEtOx 50,000 g/mol and PEtOx 100,000 g/mol as well as P*n*PrOx 50,000 g/mol. As a conclusion of these results, in general broader (but reproducible) Đ´s are observed during upscaling. A reason for the higher Đ, can be that the reactions were performed at higher temperature (60 °C vs 42 °C). Furthermore, the higher Đ´s also result from inefficient stirring which is inherent to the scale increase. In order to verify these observations, an experiment was performed to examine the influence of sampling at different places in the reaction mixture clearly revealing that stirring is insufficient at higher monomer conversions. In further development this could be improved by applying mechanical stirring. Yet, because of time constraints, this could not be tested within this PhD research.

In conclusion, this chapter describes a prosperous method for the upscaling (200 g scale) of defined high MW PEtOx and PnPrOx polymers with a range of different MW ranging from 25,000 g/mol up to 250,000 g/mol. Further optimization will be needed to reach out to other polymers in the PAOx family. Further upscaling will require the use of *e.g.* larger reactors, mechanical stirring, *etc.* In the long run, chemistry manufacturing controls (CMC) and good manufacturing practice (cGMP) facilities and procedures must be enforced to further realize the use of PAOx polymers in pharmaceutical and biomedical applications according to FDA or EMA regulations.

4.5 LITERATURE REFERENCES

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Chapter 5

CHAPTER 5

5 THE USE OF POLY(2-OXAZOLINE)S IN SOLID DISPERSIONS.

ABSTRACT Many newly developed drugs are disqualified for a validation trajectory because of their extremely low (slow) water solubility (rate) and high crystallinity. Different strategies have been targeted to overcome this low water solubility by *e.g.* the use of biocompatible polymers as excipient in so-called solid dispersions (SD). In this chapter, an overview of the results regarding the use of defined (high) molecular weight (MW) poly(2-alkyl-2-oxazoline)s (PAOx) for SD is described. SD formulations with model active pharmaceutical ingredients (APIs) having low water solubility and high crystallinity are described to demonstrate the potential of the PAOx polymer platform for oral drug formulation development. Both fenofibrate (FBT) and flubendazole (FLU) have been investigated with different formulation techniques, such as hot-melt extrusion/injection molding (HME/IM), electrospinning and solvent casting.

Contributors to this work

Glenn Verstraete & Aseel Samaro (Lab of Pharm. Tech., UGent) performance and guidance of the HME/IM work.
Ella Scholaert (Textile department, UGent) performed the electrospinning experiments with FLU.
Ali Tigrine; Master thesis student, UGent

Contribution of the candidate

Solvent casting, formulation by freeze drying and part of the HME/IM work, including guidance of the master project of Ali Tigrine, analysis and discussion of the data
Polymer synthesis (see **chapter 4**)
No manuscript in preparation.

Chapter 5

5.1 SOLID DISPERSIONS: WHAT TO DO WITH POORLY WATER SOLUBLE ACTIVE PHARMACEUTICAL INGREDIENTS?

In general, poorly water-soluble drugs or active pharmaceutical ingredients (APIs) with high crystallinity are difficult to formulate, as widely discussed in the introduction chapter (*vide* **chapter 1**). Although different formulation strategies have been described with a wide range of excipients, we will focus on polymers, especially poly(2-alkyl-2-oxazoline)s (PAOx), as pharmaceutical excipient. An interesting type of formulations are so-called solid dispersions (SD), which means that an API is dispersed in a (non-active) excipient or aiding substance, preferably the API is completely dissolved in the carrier, indicated by the absence of crystalline API fragments. The importance of having the API in an amorphous form rises from the fact that the API is trapped in a ´high energy state´, *i.e.* the energy for breaking the crystalline lattice has been overcome during formulation, which generally leads to an increase in solubility rate. The challenges for the excipient consist of preventing crystallization of the API in the formulation as well as during and after its release from the SD, eventually leading to higher absorption at the site of delivery in the body, *e.g.* the stomach or the small intestines.

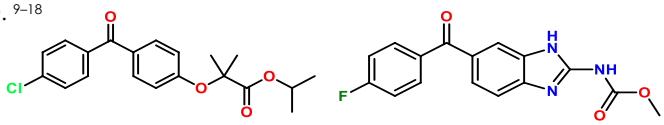
In this research chapter, formulations with PAOx and model APIs; such as fenofibrate (FBT) and flubendazole (FLU), will be highlighted and discussed. As PAOx polymers, the 50,000 g/mol poly(2-methyl-2-oxazoline) (PMeOx), poly(2-ethyl-2-oxazoline) (PEtOx) and poly(2-*n*-propyl-2-oxazoline) (P*n*PrOx) polymers, which synthesis is discussed in **chapters 3 and 4**, will be evaluated as excipient for the aforementioned APIs. Also for the FBT formulations, comparison with the ill-defined commercial PEtOx polymers, *i.e.* Aquazol®, is made since Aquazol:FBT formulations were already described in a research article by Claeys *et al.*[1] In that same year, Bender *et al.* filled a patent on the protection capabilities of Aquazol® for cannabinoids. Also, Policianova *et al*. published two papers on acetylsalisylic acid solid dispersions, including comparison between state-of-the-art excipients, such as PVP, PEG, and PEtOx polymers.[3,4] Nevertheless, it is unlikely that Aquazol® will ever be used as excipient, due to its irreproducible production process[i] which can never pass the regulatory trajectory. Therefore, the obtained results from Claeys *et al*. are considered as comparison for the behavior of defined PAOx polymers. Typical screening results will contain differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD) to gather information on the crystallinity of the API in the formulation. Additionally, dissolution studies in sink conditions are performed to show improved release kinetics compared to the bare API in solution. Both pH 1 (simulated stomach conditions) and pH 6.8 are used as dissolution medium,

[i] The Aquazol® production process is not optimized to reproduce the exact polymer structure but rather to reproduce the polymer viscosity specifications.

to simulate the release in the gastrointestinal (GI) tract and to check the influence of pH on the dissolution of the API.

FBT is a model API and class II drug in the biopharmaceutics classification system (BCS), *i.e.* it is classified as a poorly water-soluble drug with high permeability through the membrane of the stomach and small intestines. FBTs poor water-solubility originates from its high tendency to crystallize leading to a low water-solubilization rate; impairing its overall bioavailability. The fact that FBT has a low water-solubilization rate and bioavailability, makes it widely studied as a model API in formulation strategy studies. Aside from that, FBT is mainly used for the treatment of high cholesterol and triglyceride levels in the blood. Its chemical structure resembles a synthetic analogue of phenoxy-isobutyric acid derivatives (**Figure 1, left**). Currently, FBT is marketed as tablets under the trade names Tricor™ (nanocrystal; 2004), Fenoglide® and Triglide® (insoluble drug delivery microparticle; 2005) and as capsules under the trade names Antara® and Lipofen®. The main excipients nowadays used for the formulation of FBT are poly(vinyl pyrrolidone) (PVP), polyglycolized glycerides and gelatin, in the abovementioned types of formulations.[5–8]

Flubendazole is an API that belongs to the class IV drugs in the BCS system, *i.e.* it is classified as poorly water soluble and possesses low permeability through membranes in the body, thus overall poor bioavailabilty is observed. Chemically its structure is part of the benzimidazoles (**Figure 1, right**), which hold a range of active anthelmintic drugs, also known as anti-worm agents that are mostly used in veterinary science or medicine. A few commercial formulations are available under the tradenames, Flubenol, Flumoxal, Biovermin, *etc*. Mostly, excipients such as titanium dioxide, lactose monohydrate, sodium lauryl sulphate, hypromellose, PVP, PEG and brewers´ yeast are used in these flubendazole formulations. So far, enabling solid dosage forms of disclosed composition have only been reported by Vialpando *et al*. who constructed ordered mesoporous silica and spray-dried the FLU with cellulose derivatives and Vigh *et al.*, who reported very recently (appeared while our research with the electrospinning of FLU was already ongoing) the electrospinning of PVP solutions containing FLU for its immediate release from SD. [9–18]



Fenofibrate (FBT)
Class II

$S(H_2O)$ 0.000707 mg/mL
Log P 4.86
T_m 80°C

Flubendazole (FLU)
Class IV

$S(H_2O)$ 0.0334 mg/mL
Log P 2.51
T_m >= 260 °C

Figure 1. Model active pharmaceutical ingredients (APIs) used for formulation in solid dispersions, including fenofibrate (FBT, **left**) and flubendazole (FLU, **right**).

First, in **section 5.3.1**, SD with FBT will be discussed, inspired by a study by Claeys *et al.*[1] on the use of Aquazol® as matrix excipient for FBT SD, processed by hot-melt extrusion coupled to injection moulding (HME/IM). In this new study, first the influence of the possibility of using defined PEtOx polymers was investigated compared to the use of ill-defined PEtOx. Secondly, the effect of changing the hydrophilic nature of the PAOx matrix on the formulation of FBT, is investigated. Therefore, formulations with FBT and PMeOx or P*n*PrOx are compared with the formulations made with the PEtOx matrix, as a function of their immediate release capabilities of FBT. In **section 5.3.2**, the road towards FLU containing SD formulations is described using solvent casting, freeze drying and electrospinning methods, as a proof-of-concept study for its formulation. Herein, preliminary data on release kinetics of SD containing 10 % FLU and 90 % Aquazol® 200, are shown.

5.2 MATERIALS AND METHODS

5.2.1 Materials and equipment

Materials

All the defined high MW PMeOx, PEtOx and PnPrOx polymers, which are used in this chapter, are synthesized according to methods described in **chapter 2-4** and will not be further discussed here. An overview of the average number average molecular weight (M_n) is given in **Table 1**. Fenofibrate and flubendazole were purchased from Alibaba group. Aquazol® 50, 200 and 500 were kindly donated by Polymer Chemistry Innovations (PCI). The Aquazol® polymers were dissolved in water and freeze dried before use.

Table 1. PMeOx, PEtOx and P*n*PrOx polymers synthesized in chapter 2 and 4; with M_n calculated against PMMA standards and the glass transition temperatures (T_g).

Polymer name	M_n (in g/mol)	Đ	T_g (in °C)
PMeOx_50kDa	45,000	1.4	80
PEtOx_50kDa	84,700[a]	1.13	60
Aq 200	47,000	4.61	60
Aq 500	249,000	3.62	60
P*n*PrOx_50kDa	65,900	1.14	40

[a]against PEtOx standards the M_n is 50,700 g/mol.

Equipment

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50°C equipped with two PLgel 5 µm mixed-D columns in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent is *N,N*-dimethyl acetamide (DMA) containing 50 mM of lithium chloride at an optimized flow rate of 0.5 mL/min. The spectra were analyzed using the Agilent ChemStation software with the GPC add on. Molar mass and dispersity (Đ) values were calculated against polymethylmethacrylate standards from PSS.

High-performance liquid chromatography (HPLC) is performed with a Phenomenex, Luna 3u C18 (2) 100A, 50 x 2.00 mm, 3-micron column set and a guard column of Grace, Alltima C18 5u, 7.5 x 2.1mm. The column temperature and sample compartment are maintained at room temperature of 20 °C. Eluens A is H_2O + 0.1% formic acid (FA) and eluens B is acetonitrile (ACN). The flow rate was set on 0.20 mL/min, 80% A20%B, a back pressure of 102 bar with a runtime of 17 min. The injection volume used was 15 µL.

High resolution powder X-ray diffraction (XRD) was performed on a Thermo Scientific™ ARL™ X'TRA Powder Diffractometer from Thermo Fisher Scientific. The power source used is a K_α (Cu) 1.54, 40 kV and 30 mA. Furthermore, the scan type includes the 2 theta range, from 5 degrees to 70 degrees, the step size 0.02 degrees and the scan rate 1,000,000 in continuous mode.

Differential scanning calorimetry (DSC) is performed on a Mettler Toledo DSC1 Star system under nitrogen atmosphere with a heating rate of 10 °C/min from 20 °C to 200 °C (FBT containing dispersions) and 20 °C to 300 °C (FLU containing dispersions). Glass transition temperature (T_g) values are reported from the first heating run.

Hot-melt extrusion coupled to injection moulding (HME/IM) is performed on a Haake MiniLab extruder coupled to a Haake MiniJet system.

Freeze drying was performed on a Martin Christ Alpha 2-4 LDPlus with an ice condenser temperature of -85 °C and capacity of 4 kg.

Dissolution testing and drug release from the tablets is determined using the paddle method on a VK 7010 dissolution system (VanKel Industries, New Jersey, USA) with a speed of 100 rpm.

Spectrophotometric measurements were performed on a UV-1650PC system from Shimadzu Benelux.

Electrospinning set-up; All electrospinning experiments were carried out on a mononozzle setup using the solvent electrospinning technique with an 18 gauge Terumo mixing needle without bevel. A stable Taylor cone was achieved at a flow rate of 0.5 mL/h, selecting a tip-to-collector distance of 10 cm, and applying a voltage between 15-20 kV depending on the polymer concentration. It should be noted that at higher tip-to-collector distances, e.g. 20 cm, flat fibers, *i.e.* ribbons, were formed instead of round nanofibers. After electrospinning, the nanofibers were stored in an inert environment (N_2).

All nanofibrous samples were analyzed by scanning electron microscopy (SEM) using an FEI Quanta 200 FFE-SEM at an accelerating voltage of 20 kV. Samples were prepared prior to analysis by applying a gold coating using a sputter coater (Balzers Union SKD 030). The nanofiber diameters were measured using ImageJ. The average diameters and their standard deviations were based on 50 measurements per sample.

5.2.2 Experimental methods

Preparation of FBT solid dispersions

For the preparation of FBT hot-melt extrudates, a total weight of 20-30 g of the polymer:FBT mixture was mixed with a FBT content of 20 and 40 %. Prior to HME, the polymer-API mixture was cryo-milled for homogenization of the mixture. In **table 2**, the optimal process and mold temperatures are displayed, together with the pressures applied for the HME/IM experiments for each FBT:PAOx mixture. The optimal HME/IM conditions were determined according to the empiric rule of 50 °C above the T_g of the polymer used and taking into account the degradation temperature of the API. Starting from a temperature of T_g + 50 °C, adjustments of 5 – 10 °C were screened until torque values were obtained between 500 and 1000 Nm.

Table 2. Overview of the process temperatures and mold temperatures used to make FBT:PAOx SD *via* hot-melt extrusion and injection molding.

Sample Name	Polymer	Drug loading	Process Temperature	Mold Temperature	Pressure	Post pressure
SD1	PEtOx 50 kDa	20 %	115 °C	45 °C	800 bar (5 s)	400 bar (3 s)
SD2	PEtOx 50 kDa	40 %	115 °C	30 °C	800 bar (5 s)	400 bar (3 s)
SD3	Aquazol® 500	20 %	120 °C	25 °C	800 bar (5 s)	400 bar (3 s)

SD4	Aquazol® 500	40 %	125 °C	25 °C	800 bar (5 s)	400 bar (3 s)
SD5	PMeOx 50 kDa	20 %	125 °C	Not possible	Not possible	Not possible
SD6	Aquazol® 200	20 %	115 °C	25 °C	800 bar (5 s)	400 bar (3 s)
SD7	Aquazol® 200	40 %	120 °C	25 °C	800 bar (5 s)	400 bar (3 s)
SD8	PnPrOx 50 kDa	20 %	90 °C	25 °C	800 bar (5 s)	400 bar (3 s)

Preparation of flubendazole containing nanofibers

Electrospinning solutions were prepared by dissolving a specific amount of Aquazol® 200 in a 30/70 acetic acid/formic acid (AA/FA) solution (**Table 3**). Mass concentrations are expressed by weight percentages (wt%) defined by the ratio of the polymer mass and the sum of the polymer and solvent mass. For the flubendazole-containing nanofibers, a specific amount of flubendazole was first dissolved in the AA/FA solvent system after which the polymer was added. The amount of flubendazole is expressed in weight percentage (wt%) and is defined by the ratio of the flubendazole mass on polymer mass. The (dynamic) viscosity of the solutions was determined using a Brookfield viscometer LVDV-II (spindle S18, viscosity range of 1.5 - 3.0 x 10^5 cP, average error of 8%). As expected, the viscosity and nanofiber diameter increase with increasing polymer concentration.

Table 3. Overview of the viscosity and nanofiber diameter according to the Aquazol® 200 concentration and flubendazole content before and after electrospinning.

AQ200 concentration	Flubendazole concentration	Viscosity (cP)	Nanofiber diameter (nm)
20 wt%	-	1366	261 ± 84
30 wt%	-	4439	848 ± 156
20 wt%	2 wt%	1251	319 ± 92
	4 wt%	1546	192 ± 68
	6 wt%	1546	304 ± 58
25 wt%	2.5 wt%	3788	442 ± 82
	5 wt%	4394	532 ± 82
	7 wt%	4276	719 ± 108
27 wt%	3 wt%	5573	702 ± 193
	6 wt%	11413	952 ± 154

Dissolution tests

Drug release from the tablets is determined using the paddle method on a VK 7010 dissolution system (VanKel Industries, New Jersey, USA) with a speed of 100 rpm. Phosphate buffer saline (PBS) in water (pH 7) was used as dissolution medium (900 mL) at 37 °C ± 0.5 °C. Samples were withdrawn at predetermined time points (5, 10, 20, 30, 40, 50, 60, 120, 180 and 240 min) and spectrophotometrically (UV-1650PC, Shimadzu Benelux, Antwerp, Belgium) analyzed using a wavelength of 289 nm for FBT. No filtration step was included before the analysis of the UV/vis samples. For each SD type formulation three tablets were dissolved and analyzed in three separate dissolution baths, the error bars in the release profiles are provided.

For the dissolution tests of the PEtOx:FLU SD, the electrospun fibers (three times +/- 500 mg) were put in a dissolution basket as such, without additional compressing or tableting step. The analysis for the PEtOx:FLU SD was done by a custom made HPLC method, since spectrophotometric determination was impaired by the inability of setting-up a calibration curve of FLU in water due to its too low solubility. HPLC analysis is performed in water + 0.1% FA (eluens A) and ACN (eluens B), the gradient analysis is varied from A:B = 80:20 to 2:98 to 80:20. As a standard FLU and amino-FLU were used.

Statistical evaluation of the dissolution tests

Statistical evaluation was performed with Origin Pro 8.5 Statistics, using a one-way ANOVA, with Tukey's post-hoc test to assess the differences between the means of the cumulative release profiles of **Figure 6**. Significance level was chosen at a probability of $p < 0.05$, in order to assess the significance difference between the different profiles to be random or not. In the attachments (*vide* 5.6), a complete overview of the output data from ANOVA is provided.

Calibration curve FBT

A stock solution is prepared by dissolving 100.25 mg FBT in 1000 mL PBS (concentration 100.25 mg / mL). This stock solution is respectively diluted 2, 4, 6, 8 and 10 times. From these 5 samples the absorbance is measured by UV-VIS spectroscopy at 289 nm. (**Table 4**) Each measurement is performed 3 times and the average value is used in the calibration curve (**Figure 2**).

Table 4. UV/VIS spectroscopy results for the calibration curve of FBT, ranging from 10 – 50 mg/mL.

Sample	Concentration (mg / L)	Absorbance 1	Absorbance 2	Absorbance 3	Average absorbance	Standard deviation
1	10.03	0.158	0.162	0.163	0.161	0.002646
2	12.53	0.197	0.198	0.198	0.198	0.000577
3	16.71	0.258	0.262	0.263	0.261	0.002646
4	25.06	0.401	0.406	0.409	0.405	0.004041
5	50.13	0.788	0.793	0.797	0.793	0.004509

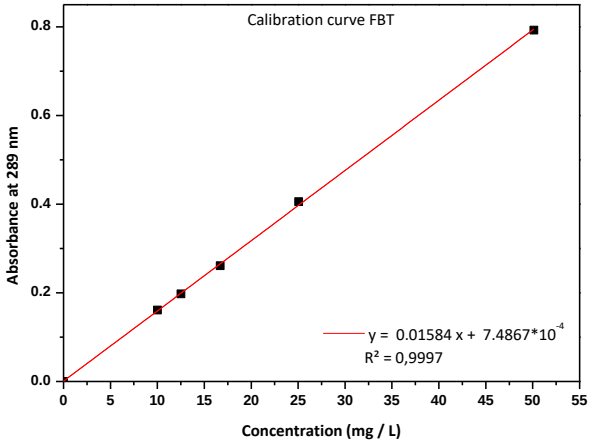


Figure 2. Standard calibration curve for FBT in PBS buffer, analysed *via* UV-VIS spectroscopy.

5.3 RESULTS AND DISCUSSION

5.3.1 Solid dispersions with fenofibrate as API

Recently, in 2012, a first study was done by Claeys *et al.*[1] on the use of PAOx polymers for SD formulation of FBT. The results (summarized in **Figure 3**) are based on Aquazol® (Aq), that is commercially available ill-defined (Đ~3-4) PEtOx, SD formulations with 20-40 % FBT content produced by HME/IM. As a first conclusion, it was found that formulations could be obtained with all the molecular weights (MW) of Aquazol®, *i.e. Aq* 50, Aq 200 and Aq 500. Secondly, no crystalline API fractions could be observed by DSC and XRD, up to 20 % FBT content. Formulations

with 40 % of FBT showed the appearance of crystalline fractions, and were not subjected for drug dissolution tests.

The *in vitro* dissolution tests of the Aq:FBT (80:20) SD showed increased dissolution compared to the pure FBT, which shows slow release up to 20-30 % after three hours (**Figure 3, right**). In contrast, the Aquazol SD with FBT show an immediate release with faster dissolution with lower MW of the Aq (Aq 500 – Aq 50) polymers.

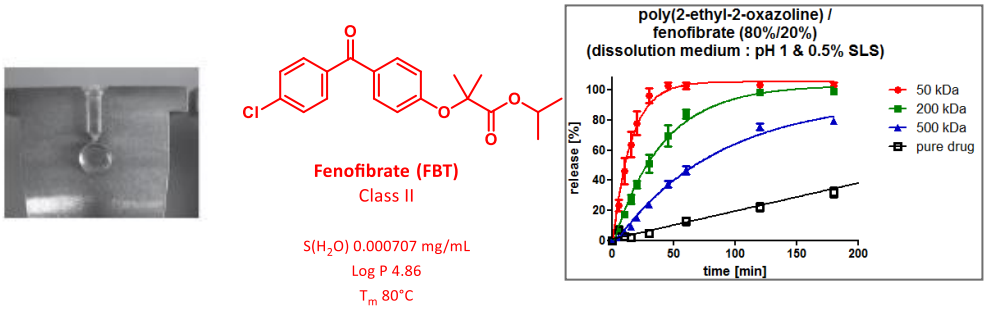


Figure 3. State-of-the-art formulation of Aquazol® with FBT processed by hot-melt extrusion/injection moulding by Claeys *et al.*[1] Injection moulded tablet after hot-melt extrusion (**left**); structure of fenofibrate, a model class II drug with very low water solubility (**middle**); and *in vitro* dissolution profile for the Aq 50, Aq 200 and Aq 500 (kDa) based SD and the release profile of the pure FBT drug. (**right**)

It has to be noted that there are some limitations of this work that are inherent to the use of Aq as excipient for SD. For example, the value of Aq for its application in SD is quite low, since its production process is not optimized to reproduce the exact polymer structure but rather to reproduce the polymer viscosity specifications. Furthermore, the polymers themselves lack a defined structure. In this particular case, Aq 50 gives the best result as excipient for the immediate release of FBT within an hour, but cannot be used as such since the Aq polymers are not made according to FDA or EMEA regulations. Additionally, the Aq 50 might be co-absorped during drug release, due to the presence of a low MW fraction.[ii] Also the Aq 200 and Aq 500 do not posses narrow specification parameters in terms of MW and Đ, making it impossible to implement them in regulated pharmaceutical formulation processes, *cfr.* current good manufacturing practice (cGMP). Therefore, as a conclusion, these preliminary data showed the potential of PAOx polymers as excipient for SD, but one can ask the question if defined PAOx polymers will display the same potential as an excipient for SD formulations, as the Aq used in the proof-of-concept study by Claes *et al.*?[1]

[ii] The absorption is limited at ± 8 nm, all polymers with a hydrodynamic radius above this limit will not be absorbed.

Hence, as a follow-up, we have examined the influence of defined PAOx on the SD formulations of FBT, produced by HME/IM. PEtOx 50,000 g/mol with Đ < 1.2 and Aq 200 and Aq 500 (**Figure 4**) have been formulated for comparison of defined versus ill-defined PAOx polymers on SD thereof.

From the tablet production process, by HME/IM, it is observed that it is more difficult to process the defined PEtOx polymer compared to the Aq grades indicating that low MW fractions in Aq make it easier to make the polymer flow in the extrusion and mold compartments. In addition, it seems that the highly crystalline FBT acts as an ´anti-plactisizer´ for the PAOx polymers. Since the FBT:PAOx formulations look more brittle compared to the pure polymer. The aq polymers are integrated in the experiments mostly as a reference towards the earlier published results.

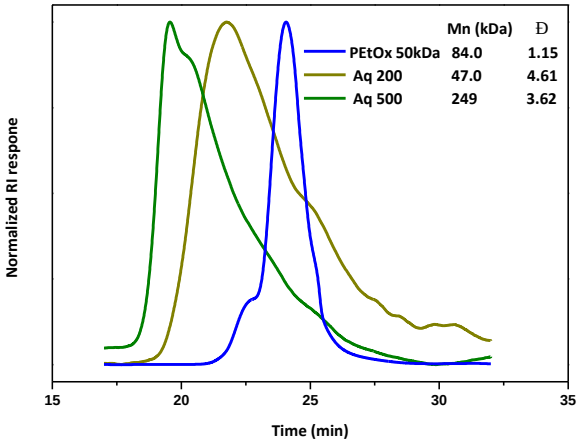


Figure 4. Size-exclusion chromatography results for PEtOx 50,000 g/mol, Aq 200 and Aq 500 polymers used in the FBT formulations. The number average MW (M_n) and Đ values are given, calculated against PMMA standards.

Characterization of the 20% FBT containing SD with PEtOx by DSC and XRD measurements (**Figure 5**) shows for all PEtOx 50,000 g/mol and Aq 200-500 formulations the absence of a crystalline fraction or crystalline structure, independent on the characterization technique which is performed.

Dissolution tests are performed to investigate the FBT release from the SD formulations according to the excipient used. In contrast to the previous tests in literature which are performed at pH 1, these tests are performed in PBS buffer (pH ~ 7). It is expected that the release will not be influenced by pH as the PEtOx matrix is highly chemically resistant, including its proven resistance against hydrolysis under acidic conditions and residence time in the stomach.[19]

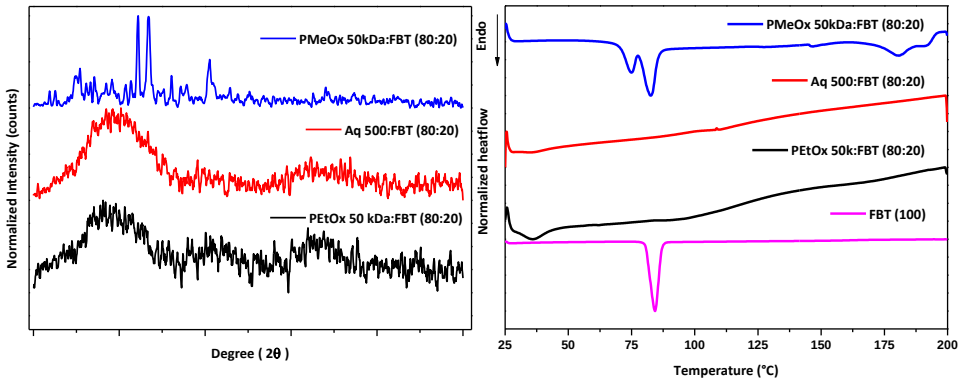


Figure 5. XRD profile of the 20 % FBT HME/IM formulations of respectively PMeOx 50,000 g/mol; Aquazol® 500 and PEtOx 50,000 g/mol (**Left**); DSC thermogram of the same FBT HME/IM formulations including the DSC of pure FBT, with a melting peak situated around 80 °C; as expected from literature. (**Right**)

This pH independence of the release is also confirmed by the immediate release profiles for the Aq polymer based tablets in **Figure 6**; which correspond well to the earlier obtained release profiles at pH 1 by Claeys *et al.*[1] The release profile for the PEtOx 50,000 g/mol matrix tablets with FBT is situated right in between the release profiles observed for the Aq 200 and Aq 500 polymers that revealed a faster and slower release, respectively. Besides this observation, statistical analysis (*via* ANOVA) does not provide any significant difference in release between the three excipients used for the formulation for FBT. Thus, it can be concluded that the use of defined PEtOx gives the same release profiles as Aq 200 and 500.

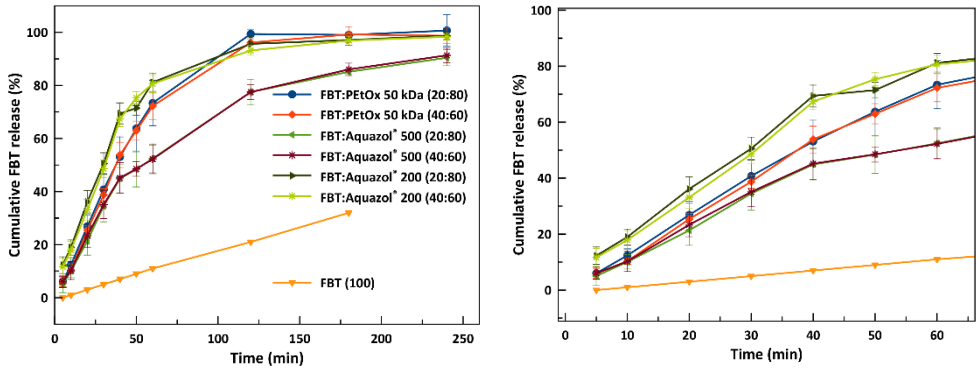


Figure 6. Results of the cumulative release of FBT from the different formulations, including pure FBT; Aquazol® 200/500 and PEtOx 50,000 g/mol. All formulations contain 20 or 40 wt% of FBT. (**left**; Zoom in for the region up to 60 min of release. (**Right**) ANOVA statistics test gives no significant difference (p = 0.05) for the means for the PEtOx and Aquazol SD of FBT (detailed description *vide* 5.6.1).

Relating to the FBT content used, it is noticed that the release curves for PEtOx:FBT 80:20 and 60:40 containing 20 and 40 % of FBT are superimposed upon each other for all polymers. This indicates that the supersaturation state of FBT is not reached. Nevertheless, partial crystallization of the FBT in the 40 % SD is observed directly after processing, by XRD and DSC, which can be harmful for the long-term stability of these formulations (**Figure 7**). Therefore, in the following experiments, it is examined if crystallization of FBT in the SD occurs over time. A SD formulation with high stability should keep the API in its amorphous form at all times. The presence of any crystalline form of the API in the SD, directly after processing, can induce further recrystallization over time.

Stability tests of the FBT:PEtOx SD are important to evaluate crystallization of the API over time, resulting in a slower and possibly incomplete release, thus lower bioavailability of the FBT. As the FBT is present in its amorphous form for the 20 % FBT formulation, recrystallization has lower chances to happen compared to the 40 % FBT SD which contains crystallized fractions of FBT already after processing. The presence of crystallinity just after processing can indicate supersaturation[iii] (*vide* **chapter 1**) of the matrix used for the preparation of the SD. Therefore, additional DSC measurements of the tablet containing the high-defined PEtOx were performed after 20 days (**Figure 7, left**). From the DSC spectrum it is clear that the tablet containing a drug load of 20% remained stable in the amorphous form, while the FBT in the formulation with a drug load of 40% completely crystallized. This could also be observed from the color change of the tablet, which turned completely opaque after 20 days (**Figure 7, right**).

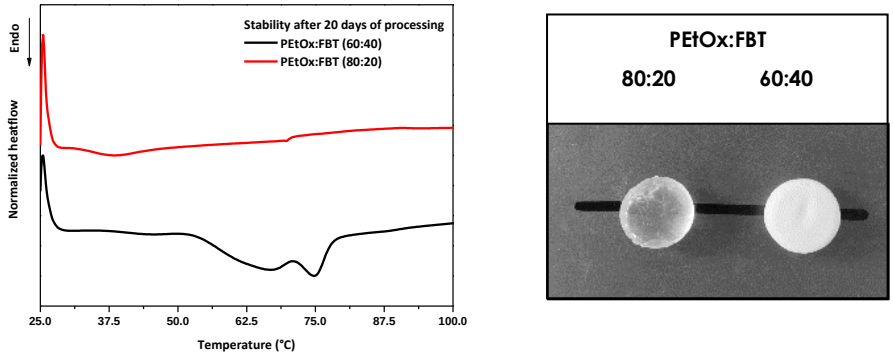


Figure 7. DSC measurement of stability tests comparing PEtOx:FBT SD formulations with 20 % and 40 % drug load after 20 days under inert atmosphere (Ar) (**Left**); Image of the IM tablets after 20 days, clearly showing recrystallization at 40 % of FBT loading. (**Right**)

[iii] Supersaturation is defined as the drug which is present in the solid dispersion at concentrations higher than the maximum concentration possible.

In order to check the influence of relative humidity on the stability (*i.e.* storage conditions), the tablets with 20 % FBT were stored under argon or air. Since the PEtOx polymers are hygroscopic in nature, the uptake of water induces lowering of the T_g, which increases the mobility of the polymer chains and the API. The improved mobility can induce faster recrystallization of the FBT in the SD. As expected, the PEtOx:FBT SD stored under air turned into a white opaque formulation indicating crystallization, while the ones stored under inert atmosphere remained their amorphous state, resulting in an absence of the crystallization peak of FBT around 75 °C (**Figure 8**).

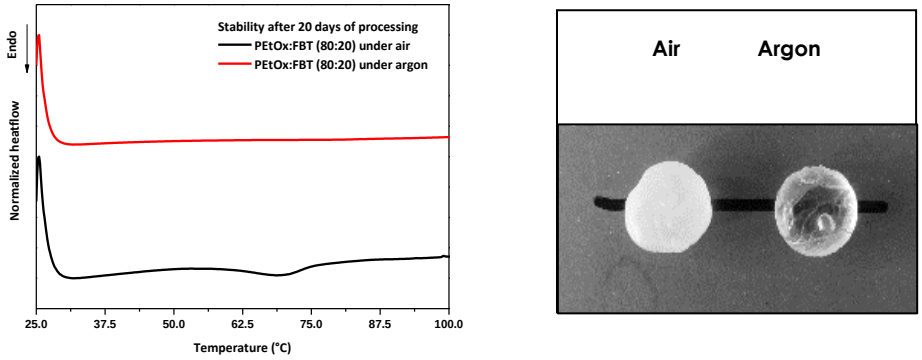


Figure 8. DSC measurement of stability tests comparing PEtOx:FBT SD formulations with 20 % drug load after 20 days under inert atmosphere and under air with a relative humidity of ± 70-80 % (**Left**); Image of the IM tablets after 20 days, clearly showing crystallization after storage under air. (**Right**)

Since the formulation results of FBT with a defined PEtOx polymer have shown the same promising and comparable results in solubility improvement of FBT as Aquazol®, we investigated if the polymer side-chain would influence the interactions with the API and if this would improve the release of FBT in SD even more. Therefore, PMeOx and P*n*PrOx 50,000 g/mol polymers were evaluated as excipient.

Unfortunately, it proved to be very difficult to formulate FBT containing formulations with PMeOx, since this matrix is most probably too hydrophilic to strongly interact with the FBT structure. The produced hot-melt extrudates were white and sticky formulations and the injection moulded tablets could not be removed from the mould, indictating recrystallatization of FBT during the formulation process. This observation was confirmed by the difference in characterization of these SD by XRD, showing a highly crystalline structure for the PMeOx:FBT SD; and by DSC where a crystalline peak (integration shows ± 72 % crystallinity) of FBT is detected at ± 80 °C (**Figure 4**). Analysis of the results of the PMeOx:FBT formulations showed poor formulations characteristics and no SD properties. Appearance of crystalline FBT may be explained by poor interactions between the hydrophilic PMeOx polymer and the more hydrophobic FBT structure.

Hence, since the PMeOx show less interactions with the FBT compared to the formulations with PEtOx, we anticipated better results from the formulation of FBT with P*n*PrOx polymers. The more hydrophobic nature of P*n*PrOx will possibly lead to better interactions with the hydrophobic FBT, compared to PEtOx. For the production of the P*n*PrOx:FBT (80:20) SD by HME/IM, the lower processing temperature of ± 90 °C was required, which was expected when taking into account a lower T_g of P*n*PrOx, which is 40 °C compared to 60 °C for PEtOx polymers. The dissolution tests from these tablets revealed, however, no improved solubility of FBT compared to the pure FBT. An overview of the results is given in **Figure 9**.

In conclusion, PEtOx 50,000 g/mol was found to be the preferred excipient for the highly crystalline FBT as model API. Related to drug content, PEtOx:FBT (80:20) SD formulations are by far the best performing. Preferably, the formulated tablets should be stored under inert atmospheric conditions and a moisture free environment due to the hygroscopic nature of the PEtOx matrix that is used as excipient.

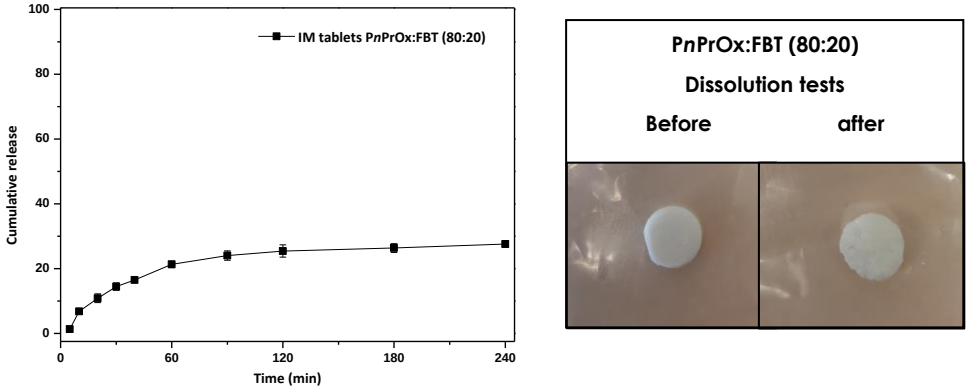


Figure 9. Dissolution tests of IM tablets of P*n*PrOx:FBT (80:20) SD formulations and combined image of the tablets before and after dissolution, clearly showing the presence of crystalline FBT. Note that the P*n*PrOx turns white when in contact with water, due to its cloud point temperature which is situated around 30 °C.

5.3.2 How can we formulate class IV APIs: Flubendazole solid dispersions

The main difficulties to formulate FLU are its high tendency to crystallize and its poor solubility in almost any organic solvent apart from formic acid and dimethyl formamide and dimethyl sulfoxide after heat treatment. It is therefore important that the excipients survive the chemical conditions for dissolution and formulation of FLU. In this aspect, PAOx polymers could serve as an ideal matrix excipient for FLU SD formulations, because of their high physical and chemical stability.

Initial experiments and trials to formulate flubendazole in an amorphous solid dispersion with PEtOx polymers as matrix excipient were performed *via* freeze drying methods. The freeze drying method is chosen primarily over solvent casting, since first experiments to perform solvent casting by mixing 5 % flubendazole and 95 % Aquazol® in formic acid failed to obtain a homogenous amorphous SD, as characterization by DSC revealed various melting peaks most likely resulting from FLU crystals (**Figure 10, right**). This freeze drying procedure included the mixing of the FLU and Aquazol® in formic acid, whereby sufficient time (*ca.* 3 days as optimum condition) is needed for homogenous dissolution of both compounds. After dissolution in formic acid, the amorphous state of FLU is expected to be kept upon fast freezing. The amorphous solution of FLU and Aquazol® is put in a petri dish and the freeze drying process is carried out in a desiccator, connected to a liquid nitrogen trap and a membrane vacuum pump (**Figure 11**).

After three days of dissolution and consequent freeze drying, the formulation showed a thermal transition at 100 °C in DSC, which can most probably be attributed to one of the different existing amorphous states or less stable crystals of FLU.[20] Because of the presence of thermal transition of FLU, we decided not to pursue any dissolution testing on these produced formulations.

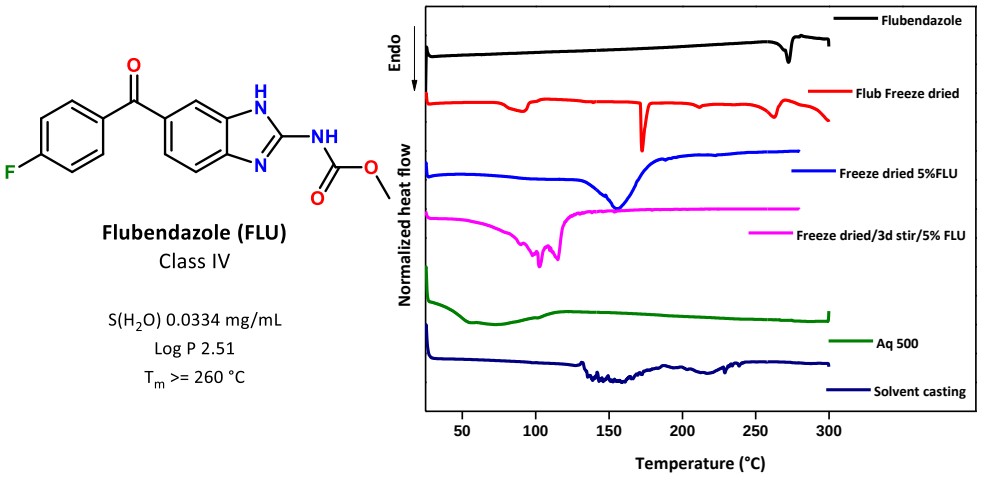


Figure 10. Structure of flubendazole (**left**) and DSC chromatograms of pure flubendazole (T_m ± 280 °C), freeze dried pure flubendazole, pure Aquazol® 500 polymer (T_g ± 60 °C) and the SD made by solvent casting and freeze drying (**right**).

In the follow-up experiments, flubendazole containing formulations were made with Aquazol®, *via* solvent electrospinning as advanced technique over spray drying, which is the main solvent method currently used for the development of SD of crystalline and poor water-soluble APIs. The idea behind using electrospinning as SD formulation development process raised from the fact that ultra-thin nanofibers can effectively and homogenously mix the FLU and

Aquazol® in a SD with efficient loss of the solvent molecules. Through the large surface area which is generated when the electrospinning process is applied, the solvent evaporates very fast hypothesized to lead to trapping of the amorphous state of FLU in the formulation.

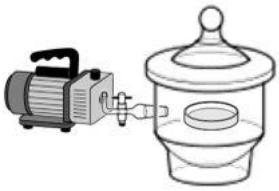


Figure 11. Example of the freeze drying experiments in dessicator, for the production of FLU:PEtOx SD.

In the following part a preliminary study is described wherein the formulation of a SD with a 10 % FLU content is evaluated. First, the parameters were optimized to electrospin solutions of PEtOx polymers up to 30 wt%, from an acetic acid/formic acid (AA/FA) solution with 30/70, 20/80 and 10/90 AA/FA ratios, of which the SEM data are shown in **Figure 12**. Here, the 30 wt% Aq 200 solutions with 30/70 and 20/80 AA/FA ratios produce the most stable nanofibers.

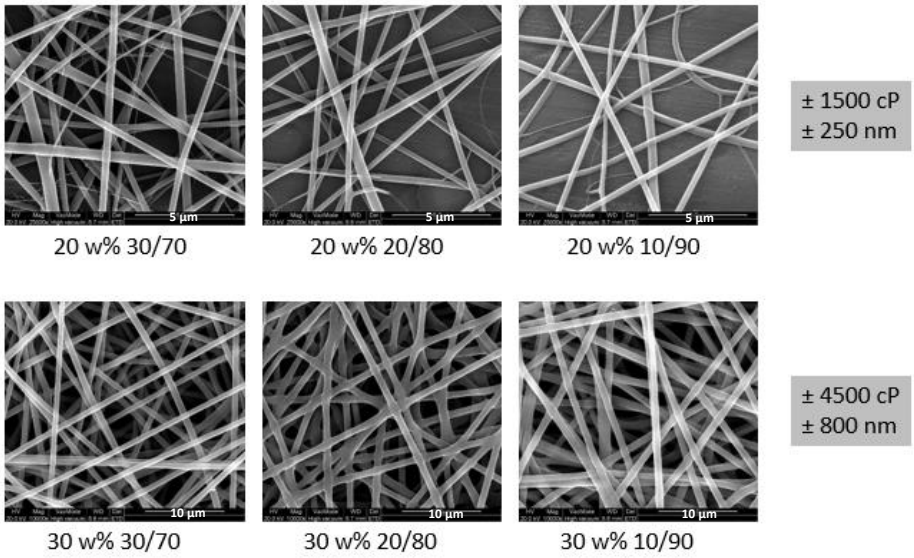


Figure 12. Scanning electron microscopy images of the different Aquazol® solutions (20 wt% and 30 wt%) electrospun from acetic acid/formic acid (AA/FA) mixtures in different ratios respectively, 30/70, 20/80 and 10/90. Yielding stable nanofibers for 30 wt% Aquazol® solutions with a 30/70 solvent mixture of AA/FA.

Secondly, a solution of 27 wt% PEtOx and 3 wt% FLU in a 30/70 AA/FA solvent mixture is chosen to prepare a FLU: Aquazol® (10:90) amorphous SD nanofibrous formulation, as recorded by SEM in **Figure 13**. Further characterization of the FLU SD was performed by DSC (**Figure 14**), showing the absence of crystallization of FLU in the PEtOx nanofibers up to three months (artefact at 240 °C also present in second and third heating run). These results have to be emphasized since these are based on a single DSC experiment taken after three months.

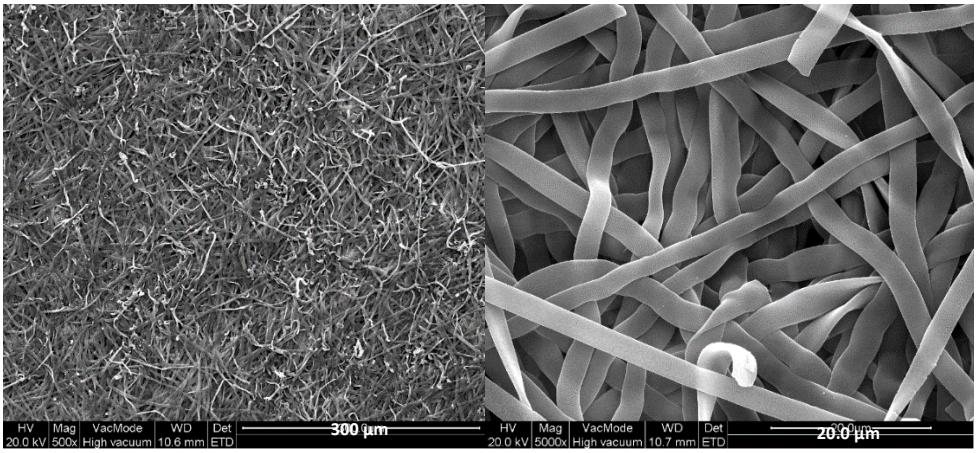


Figure 13. Scanning electron microscopy images of 27:3 wt% Aq 200:FLU (90:10) solid dispersions produced by electrospinning.

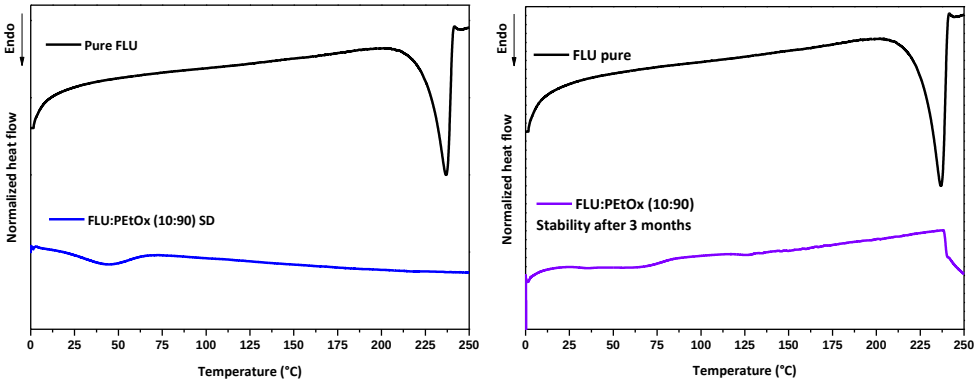


Figure 14. DSC thermograms (first heating runs) of FLU:PEtOx (10:90) SD nanofibrous formulations, after processing (**left**) and after three months of processing (**right**).

Dissolution tests on FLU:PEtOx (10:90) nanofibers

A drug dissolution test from the 10 % FLU containing SD formulation nanofibers (± 500 mg in triplicate), with Aquazol® 200 show *ca.* 80 % of FLU release after 2 hours of dissolution (**Figure**

15). From the cumulated release diagram, it appears that a supersaturated solution state is achieved around 80 % of dissolution. Since the maximum solubility of FLU is approximately 30 mg/mL and the formulation could release a maximum of 70 mg/mL theoretically. Alternatively, it could also be that the rest of the FLU is stuck in the SD. Although it is a preliminary proof-of-concept study which proofs the ability of PEtOx to maintain FLU in the amorphous form, these first test are promising for improving the bioavailability of FLU. Further development and optimization of these SD with higher drug content and the use of defined PEtOx polymers will be needed to pursue *in vivo* drug release studies and get further understanding of these formulations. The high variability of the results from **Figure 15**, could be attributed to the fact that the electrospun fibers as such were used for the dissolution tests and no further treatment was applied to make tablets. This can explain the variability of the dissolution data, because of heterogeneity in fibers/fiber mass can give difference in flotation and possible difference in the release profile. An additional milling step would homogenize and reduce the particle size with a presumed decrease in variability, which need to be tested and proven in future experiments.

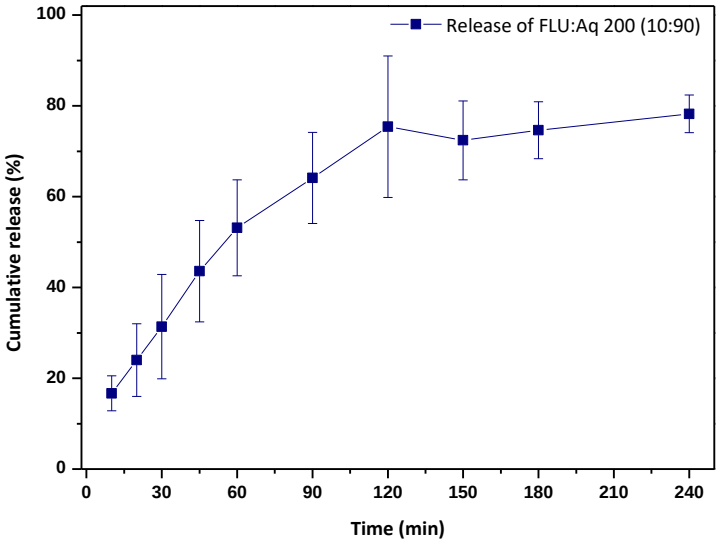


Figure 15. Dissolution test of FLU:Aquazol® 200 (10:90) SD formulations in pH 1 buffer solution (in triplicate); cumulative release analysed by HPLC method.

5.4 CONCLUSIONS AND OUTLOOK

In this chapter amorphous SD formulations with PAOx homopolymers are highlighted. Two different approaches are applied to produce such formulations, namely hot-melt extrusion coupled to injection moulding and electrospinning of nanofibrous formulations. With both techniques we showed the ability of processing PAOx polymers with poorly water-soluble APIs, in order to generate amorphous SD formulations with higher bioavailability, compared to the pure non-formulated API. More specifically we used FBT in a first case, testing PMeOx, PEtOx and P*n*PrOx with 50,000 g/mol MW for its formulation. Only PEtOx:FBT (80:20 and 60:40) SD showed an increased water-solubilization rate, while PMeOx and P*n*PrOx showed crystallinity of FBT and slow release, respectively. Short-term stability essays, 20 days after processing, only revealed good stability for the 80:20 PEtOx:FBT formulations, when preserved under inert atmosphere. In a second test case, FLU was successfully formulated in a SD with PEtOx in a proof-of-concept study using electrospinning to produce nanofibrous amorphous solid dispersions. A first trial with 10 % FLU loading and 90 % of PEtOx as excipient, showed an increased water-solubilization rate of *ca.* 80 % after 2 hours of dissolution.

In conclusion, PAOx polymers can be applied to successfully formulate some model APIs and improve their aqueous solubility rate of the APIs from these amorphous SD formulations. All of these experiments are proof-of-concept minded, showing the great potential of this PAOx polymer platform in formulation development of poorly water-soluble APIs. It will be important that the next steps in the project involve further optimization allowing the selection of the best formulation for *in vivo* experiments. Such *in vivo* data will be crucial as it is hard to predict the *in vivo* behavior based on *in vitro* dissolution tests. Once these *in vivo* data are gathered and proven to be positive, I believe that PAOx are a viable alternative as excipient for poorly water-soluble (newly developed) APIs to compete with currently marketed solubility enhancing polymers. However, it should be noted that the hygroscopic nature of PAOx, could interfere with the preparation of some SD and most of all will have an influence on the storage and stability conditions of the formulations, implying storage in moisture free conditions.

In follow-up research on the use of PAOx polymers as SD formulations, a collaboration has been started with the laboratory of Prof. Guy Van den Mooter, in order to screen different APIs and PAOx combinations with spray drying.

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5.6 ATTACHMENT

5.6.1 Figure 6: ANOVA results

ANOVAOneWay (2/06/2018 13:38:39)

Notes

Input Data

Descriptive Statistics

	Sample Size	Mean	Standard Deviation	SE of Mean
PEtOx 50kDa (20 % FBT)	10	57.537	35.83926	11.33337
PEtOx 50kDa (40 % FBT)	10	56.351	35.66049	11.27684
Aq 500 (20 % FBT)	10	46.981	30.27344	9.5733
Aq 500 (40 % FBT)	10	47.554	30.14956	9.53413
Aq 200 (20 % FBT)	10	63.155	32.21213	10.18637
Aq 200 (40 % FBT)	10	62.305	32.58461	10.30416
Pure FBT	9	9.88889	10.43365	3.47788

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	18840.04676	3140.00779	3.28854	0.00713
Error	62	59199.69722	954.83383		
Total	68	78039.74398			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.24142	0.622	30.90039	49.6787

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
PEtOx 50 kDa (40 % FBT) PEtOx 50 kDa (20 % FBT)	-1.186	13.81907	0.12137	1	0.05	0	-43.29584	40.92384
Aq 500 (20 % FBT) PEtOx 50 kDa (20 % FBT)	-10.556	13.81907	1.08028	0.98751	0.05	0	-52.66584	31.55384
Aq 500 (20 % FBT) PEtOx 50 kDa (40 % FBT)	-9.37	13.81907	0.95891	0.99336	0.05	0	-51.47984	32.73984
Aq 500 (40 % FBT) PEtOx 50 kDa (20 % FBT)	-9.983	13.81907	1.02164	0.99069	0.05	0	-52.09284	32.12684
Aq 500 (40 % FBT) PEtOx 50 kDa (40 % FBT)	-8.797	13.81907	0.90027	0.99528	0.05	0	-50.90684	33.31284
Aq 500 (40 % FBT) Aq 500 (20 % FBT)	0.573	13.81907	0.05864	1	0.05	0	-41.53684	42.68284
Aq 200 (20 % FBT) PEtOx 50 kDa (20 % FBT)	5.618	13.81907	0.57493	0.99962	0.05	0	-36.49184	47.72784
Aq 200 (20 % FBT) PEtOx 50 kDa (40 % FBT)	6.804	13.81907	0.69631	0.99887	0.05	0	-35.30584	48.91384
Aq 200 (20 % FBT) Aq 500 (20 % FBT)	16.174	13.81907	1.65521	0.9025	0.05	0	-25.93584	58.28384
Aq 200 (20 % FBT) Aq 500 (40 % FBT)	15.601	13.81907	1.59657	0.91668	0.05	0	-26.50884	57.71084

Aq 200 (40 % FBT) PEtOx 50 kDa (20 % FBT)	4.768	13.81907	0.48795	0.99985	0.05	0	-37.34184	46.87784
Aq 200 (40 % FBT) PEtOx 50 kDa (40 % FBT)	5.954	13.81907	0.60932	0.99947	0.05	0	-36.15584	48.06384
Aq 200 (40 % FBT) Aq 500 (20 % FBT)	15.324	13.81907	1.56822	0.92304	0.05	0	-26.78584	57.43384
Aq 200 (40 % FBT) Aq 500 (40 % FBT)	14.751	13.81907	1.50958	0.9352	0.05	0	-27.35884	56.86084
Aq 200 (40 % FBT) Aq 200 (20 % FBT)	-0.85	13.81907	0.08699	1	0.05	0	-42.95984	41.25984
Pure FBT PEtOx 50 kDa (20 % FBT)	-47.64811	14.19775	4.74615	0.02177	0.05	1	-90.91186	-4.38437
Pure FBT PEtOx 50 kDa (40 % FBT)	-46.46211	14.19775	4.62801	0.02746	0.05	1	-89.72586	-3.19837
Pure FBT Aq 500 (20 % FBT)	-37.09211	14.19775	3.69468	0.13997	0.05	0	-80.35586	6.17163
Pure FBT Aq 500 (40 % FBT)	-37.66511	14.19775	3.75176	0.12819	0.05	0	-80.92886	5.59863
Pure FBT Aq 200 (20 % FBT)	-53.26611	14.19775	5.30575	0.00678	0.05	1	-96.52986	-10.00237
Pure FBT Aq 200 (40 % FBT)	-52.41611	14.19775	5.22108	0.00814	0.05	1	-95.67986	-9.15237

Sig equals 1 indicates that the means difference is significant at the 0.05 level.
Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

Chapter 6

CHAPTER 6

6 THE USE OF POLY(2-OXAZOLINE)S IN SUSTAINED RELEASE FORMULATIONS.

ABSTRACT Formulation development and bioavailability improvement for good water-soluble crystalline drugs may appear counterintuitive, nevertheless they deserve similar attention compared to the aforementioned solid dispersions (SD) as discussed in **chapter 5**. Different strategies have been introduced to reduce burst drug release, *i.e.* maintain lower drug levels in blood plasma over a longer period of time, mostly improving patient compliance in terms of adverse effects, multiple dosing, *etc.* Suppression of burst release can be achieved by *e.g.* the use of biocompatible polymers as excipient in so-called extended or sustained release formulations (SRF). In this chapter, an overview of the results regarding the use of defined (high) molecular weight (MW) poly(2-alkyl-2-oxazoline)s (PAOx) for SRF are described. SRFs with metoprolol tartrate (MPT) as a model active pharmaceutical ingredient (API) with good water solubility are described, to evaluate the potential of the PAOx polymer platform for oral drug formulation development. For the preparation of the SRF, the focus of this thesis will be on hot-melt extrusion (HME) coupled to injection molding (IM) to obtain the final oral solid dosage forms. Finally, further strategies to improve the implementation of PAOx for formulation in SRF will be discussed.

Contributors to this work

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Dr. Victor R. De la Rosa; Supramolecular Chemistry Group, Ghent University

Ali Tigrine; Master thesis student

Contribution of the candidate

Manuscripts in preparation

This work serves as basis for a patent application that was filed on 16 March 2018.

Discussion of the ideas about sustained release formulations

I did the initial experiments of PnPrOx with MPT (50:50) formulations together with Ali Tigrine, as part of his master thesis (in close collaboration with Glenn Verstraete)

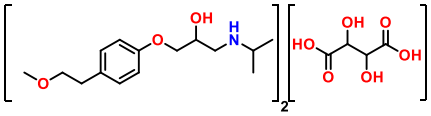
Further all experiments after the discovery of sustained release were performed by Aseel Samaro.

I contributed to the discussion of the results and analysis of the data focusing on the development of specific polymers to improve the sustained release of MPT

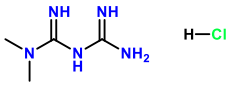
Chapter 6

6.1 THE IMPORTANCE OF CONTROLLED RELEASE: SUSTAINED RELEASE FORMULATIONS

Controlled drug delivery is omnipresent and important in current medicinal therapies, as evidenced by the large number of literature publications on this topic, including recent trends such as polymer-drug conjugates,[1–4] functionalized nanoparticles[5–8] and hydrogels.[9–17] Basically, the controlled delivery of an active pharmaceutical ingredient (API), independently of the administration route, is pursued to reduce side-effects, maintain therapeutic efficacy over a longer period of time, reduce loss of valuable API and reduce the number of administrations to increase patient compliance.[18–23] In this chapter, we will only focus on oral drug formulations, where an API and the polymer are mixed in the solid form, to serve as a sustained release formulation (SRF) for prolonged therapy. The APIs addressed in SRF mostly belong to class I in the biopharmaceutical classification system (BCS) comprising APIs that are highly water-soluble and highly permeable through the intestines, as widely discussed in chapter 1. Also class III APIs, which have high water-solubility but low permeability through the membranes could benefit from SRF, as longer time will be provided for the drug absorption in the gastrointestinal (GI) tract. If one considers oral drug administration only, formulations of model APIs such as metoprolol tartrate (MPT), [19,24–33] metformin hydrochloride (MTF)[12,22] and theophylline (THEO),[19, 22, 29,30,34,35] are typically studied for SRF based on their good water-solubility and different T_m making them representative for a wide range of other APIs (**Figure 1**). In these oral drug formulations, or so-called SRF, the drug is embedded in a preferably amphiphilic to hydrophobic matrix/excipient, while maintaining the drug in its crystalline form. In contrast to the solid dispersions (SD), described in chapter 5, an amorphous (and higher energy) state of the API, which induces faster release of the API has to be avoided at all times for the SRF. The challenges in SRF include a high drug content, tuning the release with respect to a specific API, stability and shear-resistance.



Metoprolol tartrate (MPT)
$S(H_2O)$ > 1000 mg/mL
T_m 123.6 °C
Class I



Metformin hydrochloride (MTF)
$S(H_2O)$ 50 mg/mL
T_m 230.3 °C
Class III

Theophylline (THEO)
$S(H_2O)$ 8.3 mg/mL
T_m 272.6 °C
Class I

Figure 1. Overview of the structures of metoprolol tartrate, metformin and theophylline; including their most important properties.

Recent literature highlighted the importance of biocompatible polymers with regard to SRF and other controlled drug formulations, see also **chapter 1**. One of the finest examples is the use of hydroxypropyl (methyl) cellulose (HP(M)C) matrices, which are consistently used in easy and

straightforward direct compression methods to formulate different water-soluble APIs in high dose (up to ± 50 % API).[22, 30, 32,36] Also multiple examples using hot-melt extrusion as continuous manufacturing tool for the production of SRF are found, for which some of the currently applied polymers are highly challenging due to limited processability of semi-crystalline polymers and polymers with a high glass transition temperature (T_g).[22,36,3726, 32,38] It has to be noted that the processing temperature should stay preferably below the T_m of the API, at all time during the preparation of those SRFs.

MPT is a model API and class I drug in the BCS, *i.e.* it is being classified as highly water soluble with high permeability through the membrane of the stomach and the small intestines. The high water-solubility of MPT, compared to the metoprolol succinate (MPS) salt which is often used for extended release tablets of metoprolol, probably arises from the fact that rearrangement of the tartrate salt of MP is very slow once it is dissolved, inhibiting recrystallization and favoring dissolution.[39] Pharmaceutically, MPT (and MPS) are part of the cardioselective beta blockers which are used for the treatment of hypertension and chest pain related to heart diseases such as arrhythmias. Chemically, it is a synthetic analogue of propranolol, the first clinically successful beta blocker (**Figure 2**). Nowadays MPT is commercialized as Lopressor®, whereas MPS is commercialized as Tropol XL® for which hydroxypropyl (methyl) cellulose (HP(M)C) is mainly used as binder and excipient. Up to date, no commercial formulation is available with more than 40 % drug loading.[32,40]

In a recent study by Claes *et al*., the ability of PAOx to formulate good water soluble APIs, particularly MPT, was examined. The results (summarized in **Figure 2**) are based on Aquazol® (Aq), a commercially available ill-defined (Đ~3-4) PEtOx, SRF formulations with 25 % and 50 % MPT content produced by HME/IM. As a first conclusion, it was found that formulations could be obtained with different molecular weights (MW) of Aquazol®, *i.e.* Aq 50, Aq 200 and Aq 500. Because the HME/IM parameters exceeded the melting temperature (T_m) of the MPT, the MPT was mostly present in the amorphous form; nevertheless, recrystallization was not observed *via* DSC and XRD measurements. For the Aq 500:MPT (75:25) SRF, the best results were obtained showing complete release of MPT, which was extended up to 3 hours in a pH 1 buffer solution (**Figure 2, right**). Other trends that were found include a faster release for lower MW Aq, compared to Aq 500, if the 25 % MPT loading was kept constant in the SRF. Upon increasing the MPT loading of the formulations up to 50 %, even faster release was observed compared to the 25 % MPT formulations.

In conclusion, the SRF of MPT including Aq as excipient provided promising results towards sustaining MPT up to 3 hours. Unfortunately, for the case of Aq no further fine-tuning of

the formulations seems possible since they have reached the limits of this specific matrix, in terms of drug loading and release time showing insufficiently sustained release.

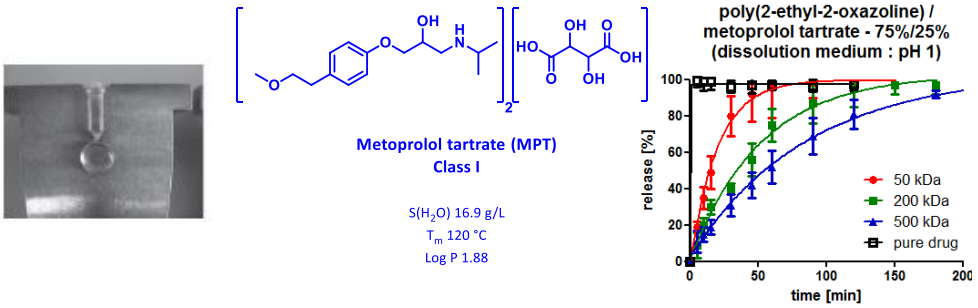


Figure 2. Injection moulded tablet after extrusion (**Left**), structure and properties of metoprolol tartrate (**Middle**) and release profile for the Aquazol SRF containing 25 wt% of MPT, in pH 1 as dissolution medium.

In this chapter, SRF with defined (dispersity, Đ, below 1.3) high molecular weight (MW) PAOx and MPT as model API, will be discussed. As PAOx polymers, a 140,000 g/mol poly(2-ethyl-2-oxazoline) (PEtOx), the more amphiphilic 50,000 - 80,000 g/mol poly(2-*n*-propyl-2-oxazoline) (P*n*PrOx) and 50,000 g/mol poly(2-*sec*-butyl-2-oxazoline) (P*sec*ButOx) polymers, prepared as discussed in **chapter 4**, will be evaluated as excipient for SRF of the highly water-soluble model API. Generally, the more hydropobic PAOx polymers are expected to give better results regarding their use in SRF. Nevertheless, if too hydrophobic polymers are used, incomplete release could be observed. The more hydrophobic PAOx used, *i.e.* P*n*PrOx and P*sec*ButOx, possess a lower critical solution temperature (LCST) behavior in water meaning that they are soluble in water at a given concentration and below a certain temperature, also known as the cloud point temperature (T_{cp}).

At first, the link between the MPT:Aquazol® SRF (studied by Claeys *et al.*[41]) and the use of defined PEtOx for its formulation *via* hot-melt extrusion coupled to injection moulding (HME/IM) is studied (**section 6.3.1**). Typical screening results will be discussed based on differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD) to gather information on the crystallinity of the API in the formulation. Additionally, dissolution studies are performed to show improved release kinetics compared to the bare API in solution. Both pH 1 (simulated stomach conditions) and pH 6.8 (simulated intestinal conditions) are used as dissolution medium, to simulate the release in the GI tract and to check the influence of pH on the dissolution of the API.

In this study, first the influence of the use of defined PEtOx polymers was questioned compared to the use of ill-defined PEtOx. Secondly, the effect of changing the alkyl side-chain of the PAOx polymers towards poly(2-*n*-propyl-2-oxazoline) leading to a more hydrophobic

nature on the ´sustained release´ profile of MPT is investigated, *vide* **section 6.3.1**. In **section 6.3.2**, SRF are prepared and investigated, containing a 50,000 g/mol P*n*PrOx polymer as matrix excipient with MPT as model API with a drug load up to 80% API (compared to the 50 % in the initial screening study in section 6.3.1). Also, the release from a 80,000 g/mol P*n*PrOx has been investigated. Finally, **section 6.3.3** will cover the use of more advanced P*sec*ButOx polymers in order to optimize the formulation of MPT, towards longer release times. Hereby, preliminary release data reveal further improvements in comparison to the earlier investigated P*n*PrOx SRF with MPT.

6.2 MATERIALS AND METHODS

6.2.1 Materials and equipment

Materials

EtOx was kindly donated by polymer chemistry innovations (PCI). BaO, HBF_4 aqueous solution, H_2SO_4, $NaHCO_3$, $MgSO_4$, MeOH, 2-phenyl-2-oxazoline, butyronitrile, ethanolamine, zincacetate and chloromethyl(trimethyl)silane were bought from Sigma Aldrich. Chlorobenzene, ethyl acetate (acroseal® grade) were purchased from thermofisher scientific. Diethylether, dichloromethane, petroleum ether and acetone were ordered at Merck. MPT was purchased from Esteve Quimica (Barcelona, Spain). The polymers were produced as discussed in **chapter 4**, see materials and methods; **section 4.2**. In **section 6.2.2**, an overview of the polymer characteristics is given in **Table 1 and 2**.

Equipment

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50 °C equipped with two PLgel 5 µm mixed-D columns in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent is DMA containing 50mM of lithium chloride at an optimized flow rate of 0.5 mL/min. The spectra were analyzed using the Agilent ChemStation software with the GPC add on. Molar mass and dispersity (Đ) values were calculated against polymethylmethacrylate standards from PSS.

Light scattering (LS) measurements are performed on a 3-angle static light scattering (MALS) detector, *i.e.* miniDAWN TREOS, from Wyatt Technology. The detector is coupled on-line to an Agilent 1260 infitiny HPLC system (*vide* DMA-SEC), and used to determine absolute molar mass

of the analyzed polymer samples. The measurements are performed at ambient temperature, i.e. no temperature control unit is supplied/installed with the above mentioned LS detector. The refractive index (RI) increment (dn/dc) values are either used as reported for the certain polymer in DMA or determined *via* online size-exclusion chromatography (SEC) equipped with an RI detector, which measures the RI increase for a 1-10 mg/mL concentration series of the mentioned polymers. The LS results are further analyzed with the provided Astra 7 software, also designed by Wyatt Technology.

High resolution powder X-ray diffraction (XRD) was performed on a Thermo Scientific™ ARL™ X'TRA Powder Diffractometer from Thermo Fisher Scientific. The power source used is a K_α (Cu) 1.54, 40 kV and 30 mA. Furthermore, the scan type includes the 2 theta range, from 5 degrees to 70 degrees, the step size 0.02 degrees and the scan rate 1,000,000 in continuous mode.

Differential scanning calorimetry (DSC) is performed on a Mettler Toledo DSC1 Star system under nitrogen atmosphere with a heating rate of 10 K/min from 20 °C to maximum 300 °C, depending on the nature of the model API which is analyzed. Glass transition temperatures (T_g) values are reported from the first heating run.

Hot-melt extrusion coupled to injection moulding is performed on a Haake MiniLab extruder coupled to a Haake MiniJet system.

Freeze drying was performed on a Martin Christ Alpha 2-4 LDPlus with an ice condenser temperature of -85 °C and capacity of 4 kg.

Dissolution testing and drug release from the tablets is determined using the paddle method on a VK 7010 dissolution system (VanKel Industries, New Jersey, USA) with a speed of 100 rpm and spectrophotometric measurements were performed on a UV-1650PC system from Shimadzu Benelux.

6.2.2 Experimental methods

Synthesis of 200 g scale poly(2-oxazoline)s at 60 °C – short description (vide chapter 4)

The synthesis of defined high MW PAOx polymers, *i.e.* 50,000 g/mol and 140,000 g/mol PEtOx; 50,000 and 80,000 g/mol P*n*PrOx and the 50,000 g/mol P*sec*ButOx is performed *via* the sacrificial initiator method (SIM) on large scale. All data regarding the polymerization, such as the amounts of initiator, monomer and solvent; and polymerization times are listed in the **table 1**

below. All polymerizations are carried out until 70 % monomer conversion. The SEC characterization data are further summarized in **table 2**.

Table 1. Overview of all reagents for the polymerization of defined high MW PAOx, on large scale.

Polymer type	MW (theoret.) (DP / kDa)	Initiator (g)	Solvent (type + mL)	Monomer (conc./ mL)	Reaction time (days)	Scale (g)
PEtOx_140kDa	1500 / 150	0.094	PhCl/ 140.5	3/ 61.2	29	60
PnPrOx_50kDa_1-3	443/ 50	1.006	PhCl/ 238.6	4.8/ 312.5	5.5	200
PsecButOx_50kDa	394/ 50	1.407	PhCl/ 190.0	4.8/ 300	6	200

Table 2. Overview size-exclusion chromatography data for the PAOx polymers used for SRF.

Batch	M_n (PEtOx)	M_p (PEtOx)	Đ	M_n (PMMA)	M_p (PMMA)	Đ
	kDa	kDa		kDa	kDa	
PnPrOx_50kDa_1	42.8	53.8	1.14	66.6	80.5	1.11
PnPrOx_50kDa_2	40.0	44.9	1.13	61.3	80.2	1.18
PnPrOx_50kDa_3	45.1	56.1	1.16	69.8	83.4	1.12
PEtOx_140kDa	117.5	141.5	1.35	181.8	206.7	1.32
PEtOx_Aquazol_200	16.5	170.5	9.00	36.1	174	4.80
PEtOx_Aquazol_500	53.1	937.3	8.30	120	496	4.10
PnPrOx_80kDa	40.9	61	1.40	72	99.4	1.30
PsecButOx_80kDa	27.3	40,0	1.37	40.6	71.3	1.40
PsecButOx_50kDa	13.2	18.4	1.26	25.6	35.0	1.22

Preparation MPT:PEtOx, PnPrOx (50:50), MPT:PnPrOx (70:30 or 80:20) and MPT:PsecButOx (70:30 or 80:20) tablets via HME/IM

For the preparation of the MPT SRF, first the pre-weighted mixture of API and polymer excipient is cryo-milled to homogenize before HME/IM. Every mixture contained in total a tablet weight of around 500 mg per tablet; and three identical tablets were chosen for sequential dissolution tests. In **Table 3**, an overview is given of all the HME/IM (**Figure 3**) parameters. The processing temperature for the extruder varied between 110 – 145 °C, depending on the

polymer type used. The mold temperature depicts the IM settings, between 25-30 °C, and the pressures applied during the process and after processing are respectively 800 bar for 10 s and 400 bars for 5 s. Post-pressure is generally applied to prevent the tablets from falling apart, due to elastic recovery, after processing with such high pressures.

Figure 3. Twin-screw co-rotating hot-melt extruder (**Left**) and high pressure injection moulder with adjustable temperature set(**Right**) that was used to prepare the SRF.

Table 3. Processing temperatures for HME/IM, for the production of MPT:PAOx SRF.

SRF	Drug load %	HME Temperature °C	IM Temperature °C
MPT:Aq200	50	130	135
MPT:Aq500	50	145	145
MPT:PEtOx_140kDa	50	130	135
MPT:PnPrOx_50kDa	50	110	130
MPT:PnPrOx_50kDa_1-3	70 - 80	110	130
MPT:PsecButOx_50kDa	70 - 80	110	110-120

All tablets contain in total approximately 500 mg of material, including API and excipient, and the drug content is varied between 96.6 and 103 % of the desired value, *e.g.* in a MPT:P*n*PrOx (70:30) SRF, the 70 wt% of API is achieved within 96.6 and 103 %.

UV/Vis Calibration curve MPT

A stock solution is made by dissolving 100.5 mg MPT in 200 mL of PBS (concentration = 502.5 mg / L). This stock solution is diluted respectively 4, 6, 8, 10, 20 and 40 times. From these six samples the absorbance is measured by UV-Vis at a wavelength of 274 nm (**Table 5**). Each measurement is performed three times and the average values are used in the calibration curve (**Figure 4**).

Table 5. Absorption curve data for the calibration curve of MPT.

Sample	Concentration (mg / L)	Abs 1	Abs 2	Abs 3	Average abs	Standard deviation
1	12.56	0.062	0.062	0.063	0.062	0.000577
2	25.13	0.116	0.117	0.116	0.116	0.000577
3	50.25	0.195	0.195	0.193	0.194	0.001155
4	62.81	0.250	0.247	0.250	0.249	0.001732
5	83.75	0.334	0.330	0.330	0.331	0.002309
6	125.63	0.507	0.506	0.507	0.507	0.000577

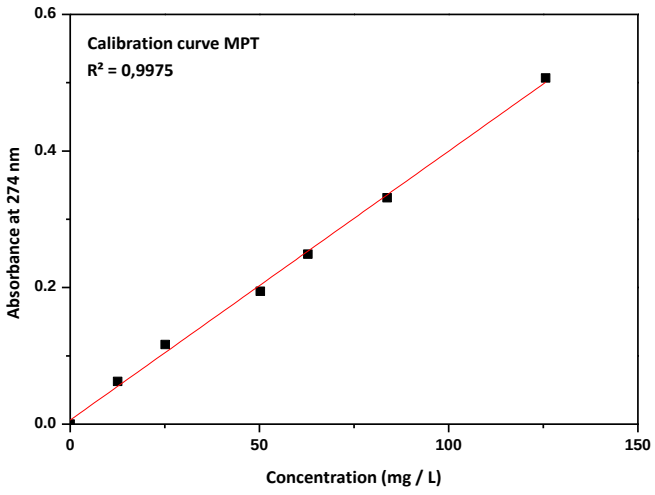


Figure 4. Calibration curve of MPT, measured *via* UV/VIS spectroscopy at a wavelength of 274 nm.

Dissolution tests

Drug release from all the prepared tablets is determined by using the paddle method on a VK 7010 dissolution system (VanKel Industries, New Jersey, USA) with a speed of 100 rpm. Simulated intestinal fluid (SIF, pH 6.8) and PBS were used as dissolution medium (900 mL) at 37 °C ± 0.5 °C. Samples were collected at predetermined time points (30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 16 h, 20 h and 24 h) and spectrophotometrically (UV-1650PC, Shimadzu Benelux, Antwerp, Belgium) analyzed using a wavelength of 274 nm for MPT. No filtration step was included before the analysis of the UV/vis samples. For each SRF type formulation three tablets were dissolved and analyzed, in three separate dissolution baths, the error bars in the release profiles are provided.

Statistical evaluation of the dissolution tests

Statistical evaluation was performed with Origin Pro 8.5 Statistics, using a one-way ANOVA, with Tukey's post-hoc test to assess the differences between the means of the cumulative release profiles of figures 7, 9, 10 and 12. Significance level was chosen at a probability of $p < 0.05$, in order to assess the significance difference between the different profiles to be random or not. In the attachments (*vide* 6.6), a complete overview of the output data from ANOVA sorted per figure.

Disintegration tests

Disintegration tests were performed as per Pharmacopoeia standards.[42] The disintegration time was tested on three different tablets from the same formulation batch using Pharma test disintegration tester (Dist-3, Germany) with discs. PBS was used as disintegration medium and the temperature was set at 37 °C. The time was recorded until no material from any of the tablets was left on the mesh.

6.3 RESULTS AND DISCUSSION

6.3.1 Initial tests with Aquazol® and defined high molecular weight poly(2-oxazoline)s for sustained release formulations

In this study, we first questioned if the use of defined high MW PEtOx, with a MW of 140,000 g/mol, would be feasible in SRF with MPT and if it would further sustain the release (compared to the results with Aq in **Figure 2**). Secondly, additional assets to change the side-chain structure of the PAOx polymers towards a more hydrophobic moiety, *e.g.* towards P*n*PrOx 50,000 g/mol, are proposed for the preparation of extended SRF in combination with MPT. An overview of the size-exclusion chromatography (SEC) results for these polymers used as matrix for the MPT SRF can be found in **Figure 5**.

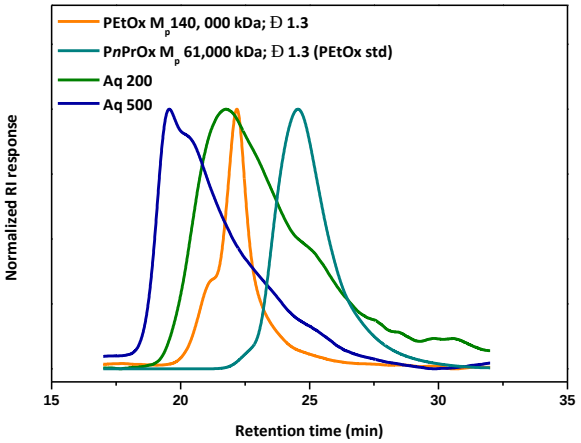


Figure 5, Size-exclusion chromatograms (SEC) of the Aquazol, defined PEtOx and P*n*PrOx polymers used for the preparation of SRF containing 50 wt% MPT. SEC is measured in *N,N*-dimethylacetamide with LiCl, while MW results are calculated against PEtOx standards.

Tablets produced by HME/IM revealed a stringent difference between the PEtOx and P*n*PrOx matrices for SRF. For the 140,000 g/mol **PEtOx:MPT (50:50) SRF** the tablets were found to be glassy and transparent immediately after processing. Since the processing temperature of 130 °C resembles the T_m of MPT, MPT is present mostly in the amorphous form, explaining the appearance of the tablets after preparation. However, for SRF it is better to keep the API in the crystalline form to assure extended release. Efforts to lower the processing temperature of the extrusion process for this PEtOx matrix towards 100 °C failed and resulted in a too high torque. The production of ill-defined, Aq:MPT (50:50) SRF, was also repeated as previously reported by Claeys *et al.*;[41] and used as a reference for the characterization and dissolution experiments.

In contrast to PEtOx, the production of the 50,000 g/mol **P*n*PrOx:MPT (50:50) SRF** resulted in white and rather sticky tablets. The white color indicates that the MPT is present in its crystalline form, which will be confirmed in further characterization experiments, as discussed later in this section. The stickiness of the tablets, on the other hand, originates from the characteristics of the P*n*PrOx polymer used, *i.e.* a low glass transition temperature of approximately 40 °C. The process temperature for these SRF could be set at a lower value of 110 °C compared to PEtOx SRF keeping the MPT crystalline.

Characterization of the PEtOx:MPT and P*n*PrOx:MPT (50:50) by XRD and DSC (**Figure 6**) confirmed the visual observation after production of both types of tablets. The PEtOx:MPT tablets show no crystallinity, for the Aquazol polymers, and minor crystallinity (± 15 %), for the formulation with the 140,000 g/mol PEtOx polymer, respectively. Whereas the P*n*PrOx SRF with MPT revealed a crystalline structure in XRD and the melting transition in DSC confirms the obtained white tablets to be crystalline.

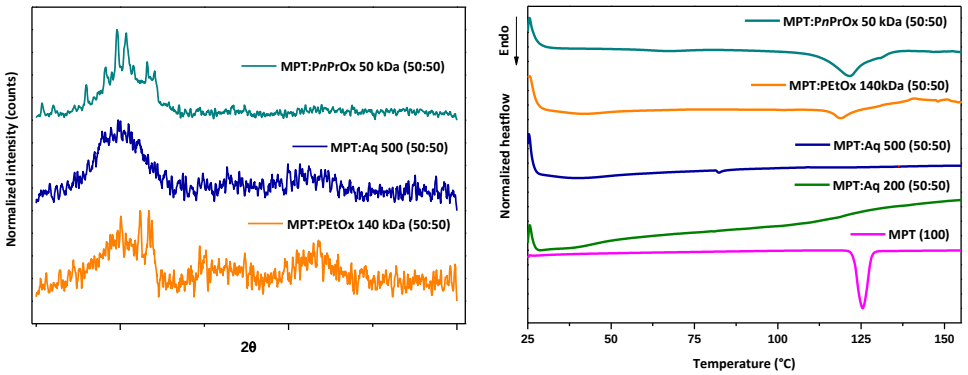


Figure 6. XRD (**Left**) and DSC (**Right**) characterization data for the PEtOx:MPT and P*n*PrOx SRF with 50 wt% of MPT. As a reference the DSC of pure MPT is presented.

Dissolution tests are performed for the PEtOx:MPT and P*n*PrOx:MPT (50:50) SRF in a PBS (pH 6.8) buffer solution and release of the MPT concentration was measured *via* UV-Vis spectrometry at an absorption wavelength of 274 nm. Prior to the dissolution tests, a MPT calibration curve was established, which can be found in **section 6.2.2**. The dissolution medium for all the tablets is identical and tests were performed in triplicate, for accurate analysis. For the PEtOx:MPT tablets, an experiment timeframe of 6 hours was chosen, since the release is expected to be analogous to the Aquazol:MPT tablets measured in the previous study by Claeys *et al.*[41] For the P*n*PrOx:MPT tablets a timeframe of 24 hours was chosen as no release studies were ever carried out for this excipient. The results of the dissolution tests can be found in **Figure 7**.

Similarly to the earlier release studies by Claeys *et al.* (**Figure 2**),[41] complete release from the Aquazol® matrices is observed after only two hours, with Aq 500 showing a slower sustained release profile compared to the Aq 200 matrix. For the 140,000 g/mol PEtOx:MPT SRF very similar release is observed as with Aq 200 indicating that further increasing the MW of the PEtOx polymer, will most likely not result in the desired difference in the sustained release capacity of the PEtOx matrix, *i.e.* preferably over 24 hours. A one-way ANOVA statistical analysis revealed that the variations in data for the Aq 500, Aq 200 and PEtOx 140kDa formulations are rather random, *i.e.* not significantly different for a 0.05 confidence level.

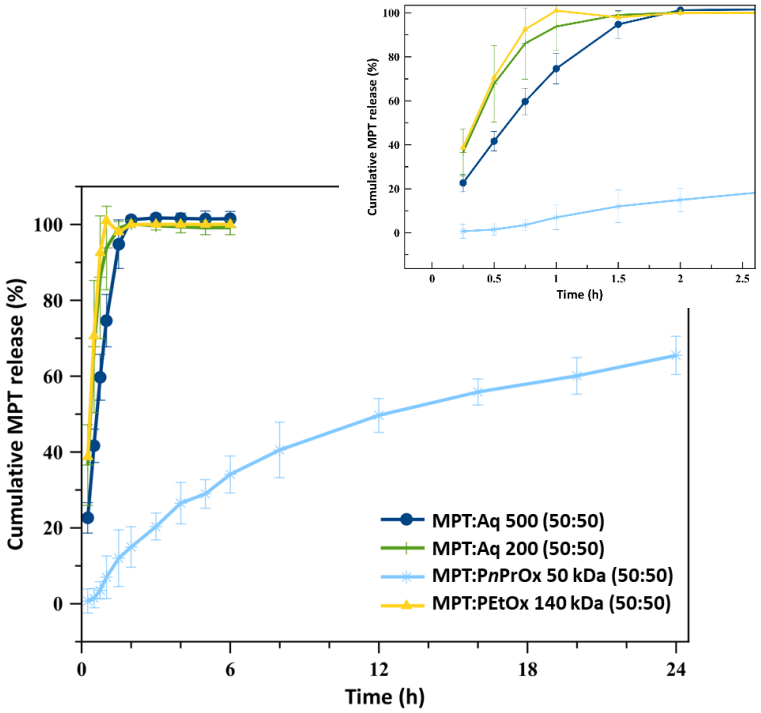


Figure 7. Dissolution testing for the SRF of Aquazol, 140,000 g/mol PEtOx and 50,000 g/mol PnPrOx containing 50 wt% of MPT; in PBS (pH 6.8). ANOVA statistics test gives a significant difference ($p = 0.05$) for the means, MPT:P*n*PrOx is significantly different from the other data sets (detailed description *vide* 6.6.1).

For the 50,000 g/mol P*n*PrOx:MPT (50:50) SRF, the sustained release profile changed drastically, with only 60 % cumulated release of the MPT after 24 hours. Despite the fact that we expected the release to be more sustained by changing the side-chain from ethyl towards propyl, such a large increase in release time was rather surprising and shows a huge potential in the development of SRF with MPT and other APIs. Statistical analysis *via* one-way ANOVA confirmed that the data for P*n*PrOx 50 kDa are significantly different from the release profiles with Aq and PEtOx polymers.

6.3.2 Sustained release formulations with poly(2-*n*-propyl-2-oxazoline) polymers and metoprolol tartrate

As a follow up on the promising and preliminary results concerning the P*n*PrOx 50,000 g/mol polymer as matrix excipient in SRF containing 50 wt% of MPT (section 6.3.1), the questions arose whether drug loading could further be increased and whether the polymer molecular weight influences the release profile, if other APIs could form SRF with P*n*PrOx and which methodologies could be applied for the formulation process and consequent tableting method. In this section, the increased drug loading and the influence of molecular weight on the drug release will be discussed with regard to HME/IM of MPT:P*n*PrOx SRF, while the other questions are being addressed by Aseel Samaro in the framework of her PhD at the Faculty of Pharmacy (Ghent University).

To push the limits of the SRF and to challenge the formulation to its maximum drug capacity in terms of release profile, formulation of MPT with P*n*PrOx 50,000 g/mol was performed with a drug loading that was increased up to 70 % and 80 % of MPT (**Figure 8**). Since, MPT is regarded as one of the most hydrophilic and low melting model API, these target drug loads are quite challenging as will be demonstrated by the dissolution profiles.

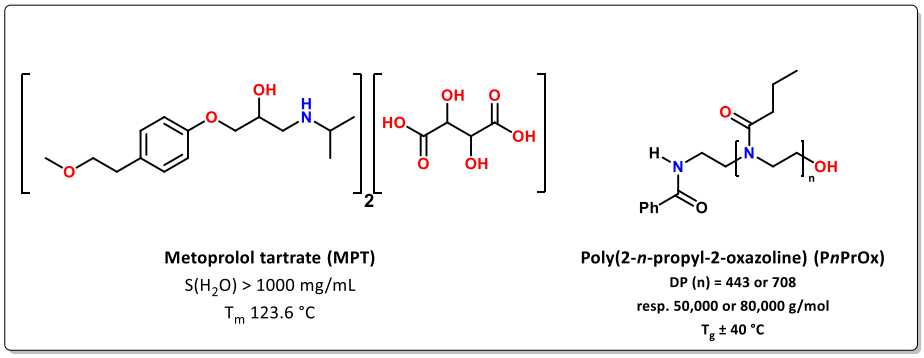


Figure 8. Overview of the structures of MPT; including its most important properties, *i.e.* water solubility and melting temperature T_m, in combination with the structure of poly(2-*n*-propyl-2-oxazoline) 50,000 and 80,000 g/mol.

MPT:PnPrOx (70:30)and (80:20) sustained release formulations

The dissolution profile of the prepared MPT:P*n*PrOx (70:30) SRF prepared *via* HME/IM can be found in **Figure 9**. Complete release is achieved after 6 hours, in contrast to the incomplete release profile found up to 24 hours for 50 % of MPT with P*n*PrOx as matrix excipient. Not only MPT dissolution was tested, but also the mechanical strength of the tablets was evaluated with a disintegration test. During the disintegration test, the tablet is moved up and down in the

dissolution medium, to simulate the peristaltic movements of the GI tract *via* an *in vitro* test. Disintegration was found to be faster, *i.e.* less than an hour, than the provided release profile in **Figure 9** posing a potential problem for further use. A reason for this behavior is most probably attributed to the fact that only 80 % of crystalline MPT is found in the IM tablets, which are processed ($T_{process}$ = 130 °C) around the T_m of MPT (123 °C) inducing partial melting of the MPT. Furthermore, the P*n*PrOx matrix possesses a low glass transition temperature, around 40 °C, which makes the tablets prone to easy deformation.

Increasing the drug load up to 80 % MPT (**Figure 9**) accelerates the release even more up to 4 hours. As an intermediate conclusion for MPT SRF with P*n*PrOx as matrix excipient, incomplete release was found with IM tablets containing 50 % of MPT compared to full release after 4 hours with 80 % of MPT loaded into the tablets. Even though a more ideal sustained release may be anticipated at 60 % of MPT, all IM tablets showed poor disintegration stability, making the P*n*PrOx matrix non-viable to apply them for SRF of MPT.

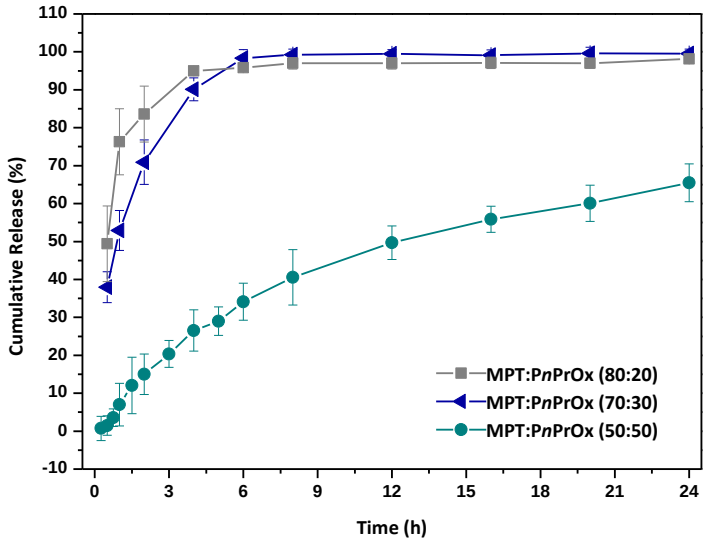


Figure 9. Release profile for the MPT:P*n*PrOx (50:50, 70:30 and 80:20) SRF in simulated intestinal fluid (SIF, pH 6.7) up to 24 hours. ANOVA statistics test gives a significant difference (p = 0.05) for the means, MPT:P*n*PrOx (50:50) is significantly different from the other data sets with MPT:P*n*PrOx 70:30 and 80:20 (detailed description *vide* 6.6.2).

Statistical analysis of **Figure 9,** *via* one-way ANOVA, shows that the 50 % MPT formulations with P*n*PrOx are significantly different from the 70 and 80 % formulations. The data for the MPT:P*n*PrOx (70:30) and MPT:P*n*PrOx (80:20) are not significantly different.

Based on these results two strategies are proposed to provide more mechanical stability to the tablets. First in this section, the influence of molecular weight of the P*n*PrOx matrix

on the release profile and disintegration stability will be investigated. The second strategy holds changing the polymer structure towards a hydrophobic polymer with a higher T_g, which will be further elaborated in **section 6.3.3**.

In an attempt to overcome the low disintegration stability, P*n*PrOx with a MW of 80,000 g/mol was investigated for SRF with 70 % MPT. In the comparison of the SRF based on the 80,000 g/mol P*n*PrOx polymer, with the 50,000 g/mol P*n*PrOx the release was found, to be slower (but not significant according to ANOVA) with the larger polymer *i.e.* 8 hours versus 6 hours (**Figure 10**). Even though the release form the higher MW P*n*PrOx was slightly longer sustained, no significant improvement was found in the disintegration test. Therefore, it can be concluded that the search for another more, hydrophobic PAOx polymer (see **section 6.3.3**) is the preferred way in the search for an optimal SRF with MPT.

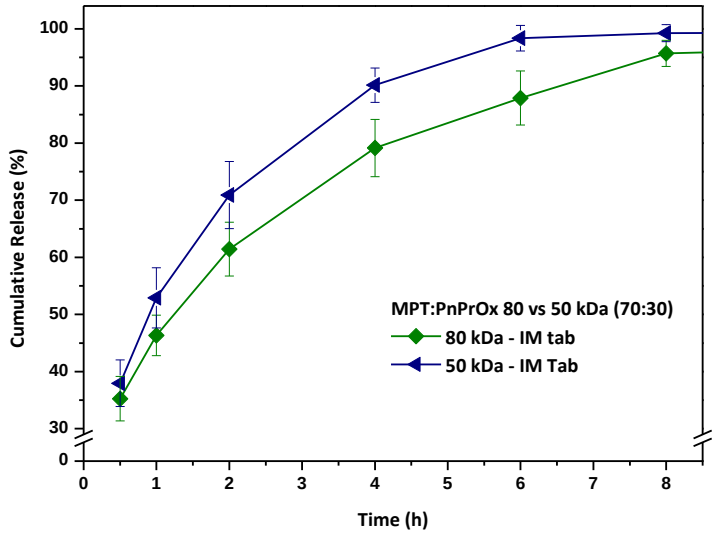


Figure 10. Release profile for the MPT:P*n*PrOx (70:30) SRF in simulated intestinal fluid (SIF, pH 6.7) up to 8 hours, with different MW, *i.e.* 50,000 g/mol vs 80,000 g/mol, P*n*PrOx polymers as excipient. ANOVA statistics test gives not a significant difference (p = 0.05) for the means (detailed description *vide* 6.6.3).

6.3.3 Sustained release formulations with poly(2-sec-butyl-2-oxazoline) polymers and metoprolol tartrate

An important parameter for the mechanical stability of the tablets, at a certain temperature, is the T_g. Since the T_g of P*n*PrOx is quite close to room temperature, *i.e.* approximately 40 °C, the P*n*PrOx behaves as a fragile matrix with regard to tablet disintegration studies at room temperature, *vide supra*. The proposition for another, higher T_g polymer with

preferably the same or stronger hydrophobic properties than P*n*PrOx pointed us in the direction to synthesize a 50,000 g/mol P*sec*ButOx polymer (**Figure 11**).

The T_g of P*sec*ButOx is situated around 55 °C, which should give enough mechanical strength at room temperature in its usage as a matrix excipient, to prevent fast disintegration of tablets made thereof. Therefore, SRF of MPT were studied using a 50,000 g/mol P*sec*ButOx polymer as sustained release matrix. For a good comparison, the release profile of the API:P*n*PrOx SRF are shown in comparison to the new release profiles (**Figure 12**).

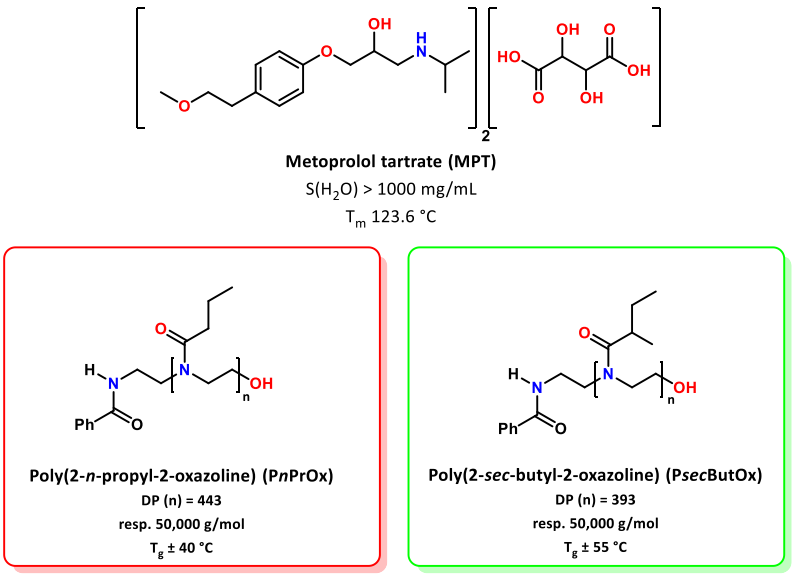


Figure 11. Overview of the structures of the most hydrophilic model APIs, including MPT and MTF; including their most important properties, *i.e.* water solubility and melting temperature T_m, in combination with the structure of poly(2-*n*-propyl-2-oxazoline) and poly(2-sec-butyl-2-oxazoline) both with a MW of 50,000 g/mol.

MPT:PsecButOx (70:30)and (80:20) sustained release formulations

The results for the MPT:P*sec*ButOx SRF are shown in **Figure 12**. The slower release of P*sec*ButOx SRF compared to the P*n*PrOx matrix formulations immediately stands out. While the P*n*PrOx matrix led to complete MPT release after 6 hours, only 60 % of MPT release is achieved after 24 hours with the P*sec*ButOx matrix. Importantly, the processing temperature for IM could be kept at 110 °C for P*sec*ButOx, which induced no loss of crystallinity of MPT in the SRF. The latter could be an explanation why the release is so different compared to the previous observed release profiles for P*n*PrOx. Furthermore, P*sec*ButOx is more hydrophobic and possesses a higher T_g, in comparison to the P*n*PrOx matrix, which can also contribute to longer retention of MPT. The

disintegration tests show overall better performance for the P*sec*ButOx polymers compared to P*n*PrOx, as no shear-stress instability is observed within a time frame of 24 hours.

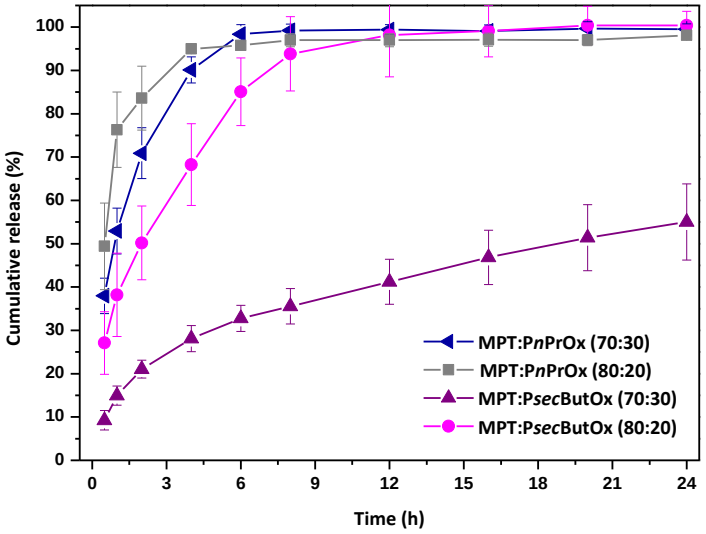


Figure 12. Release profile for the MPT:P*sec*ButOx (80:20 and 70:30) SRF in simulated intestinal fluid (SIF, pH 6.7) up to 24 hours, with comparison of P*sec*ButOx and P*n*PrOx (MPT:P*n*PrOx; 80:20 and 70:30) polymers as excipient. ANOVA statistics test gives a significant difference ($p = 0.05$) for the means, MPT:P*sec*ButOx (80:20) is significantly different from the other data sets (detailed description *vide* 6.6.4).

The SRF of P*sec*ButOx with a drug load of 80 % of MPT showed complete release after approximately 9 hours compared to only 4 hours for P*n*PrOx as matrix for SRF. Altogether, it can be concluded that a tremendous improvement of the tablet properties with P*sec*ButOx as matrix for the formulation of MPT is achieved as no disintegration was found for this 80 % MPT SRF either, which is highly promising towards future *in vivo* experiments. In the statistical analysis, *via* ANOVA, it is found that only the MPT:P*sec*ButOx (80:20) is significantly different from the other data sets. Although statistical analysis is provided, we have to be careful with the drawn conclusions. For example if we include the disintegration data, MPT:P*sec*ButOx (70:30) is also significantly different from the P*n*PrOx formulations, since the disintegration time is found to be 40 min for MPT:P*n*PrOx (70:30) and for MPT:P*sec*ButOx (70:30) 12 hours.

6.4 CONCLUSIONS AND OUTLOOK

In this final chapter, the formulation of MPT with the use of PAOx polymers, more specifically PEtOx, P*n*PrOx and P*sec*ButOx, as matrix excipient for SRF, is discussed.

Firstly, the formulation of a 50 wt% containing MPT SRF with Aquazol, 140,000 g/mol PEtOx and 50,000 g/mol P*n*PrOx was formulated *via* HME coupled to IM, as a proof-of-concept study. Complete characterization, by DSC and XRD, revealed the presence of only a minor crystalline fraction of MPT in the PEtOx matrices, whereas high crystallinity of MPT was found in the P*n*PrOx. The latter observation was verified by performance of dissolution tests on all the prepared tablets, revealing fast release from the PEtOx matrices compared to sustained release of only 60 % after a period of 24 hours for the P*n*PrOx matrix. Despite the fact that we expected a change in dissolution profile of the MPT when going from a hydrophilic matrix, PEtOx, towards a thermo-responsive and more hydrophobic matrix, P*n*PrOx, it is undoubtedly a drastic change in release profile.

As a second part of this chapter, further investigations were performed to evaluate P*n*PrOx as matrix for formulating higher drug loads of MPT, combined with analysis of the MPT SRF with P*n*PrOx of varying molecular weight and tablet stability. For the MPT:P*n*PrOx SRF a drug load between 50 and 80 % revealed sustained release within 24 hours down to 4 hours, respectively. However, the shear-stress stability, *i.e.* the ability to disintegrate, of the P*n*PrOx containing SRF with MPT was insufficient as the tablets did not pass the disintegration test within the time period where they provide release of the specific API. In other words, the tablets would not survive appropriate *in vivo* shear-stress conditions and consequently may induce faster release compared to what is shown in the *in vitro* dissolution tests, also when a higher MW P*n*PrOx of 80,000 g/mol was used.

In a final part, P*sec*ButOx was proposed as an alternative for the P*n*PrOx matrix, to formulate MPT. The P*sec*ButOx is slightly more hydrophobic and possesses a higher T_g, resulting in higher tablet stability compared to the use of the P*n*PrOx matrix. The release pattern for MPT changed to 60 % release after 24 hours for a drug load of 70 % of MPT and complete release after 9 hours when a drug load of 80 % was used. These preliminary results in combination with the higher shear-stress stability indicate that these P*sec*ButOx tablets have great potential for SRF and studies will be extended to *in vivo* in the near future.

As a conclusion, this chapter provides a case study for the use of thermo-responsive and hydrophobic PAOx polymers as matrix excipient for the formulation of SRF with an extremely water-soluble model API, *i.e.* MPT. It will be important on short-term, to evaluate all the data which are gathered *via in vitro* dissolution tests and to select the most promising SRF for further analysis in *in vivo* tests.

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6.6 ATTACHMENTS

6.6.1 Figure 7: ANOVA results

ANOVAOneWay (2/06/2018 12:21:49)

Notes

Input Data

Descriptive Statistics

	Sample Size	Mean	Standard Deviation	SE of Mean
Aq500:MPT	10	80.094	29.30224	9.26618
Aq200:MPT	10	88.074	20.74552	6.56031
PnPrOx:MPT	15	28.09067	22.1163	5.7104
PEtOx140kDa:MPT	10	90.085	20.2597	6.40668

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	34191.13574	11397.04525	21.10286	1.99817E-8
Error	41	22142.91302	540.07105		
Total	44	56334.04876			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.60694	0.34814	23.23943	66.75311

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
Aq200:MPT Aq500:MPT	7.98	10.39299	1.08587	0.86844	0.05	0	-19.84848	35.80848
PnPrOx:MPT Aq500:MPT	-52.00333	9.48746	7.75169	1.3709E-5	0.05	1	-77.40714	-26.59952
PnPrOx:MPT Aq200:MPT	-59.98333	9.48746	8.9412	8.79567E-7	0.05	1	-85.38714	-34.57952
PEtOx140kDa:MPT Aq500:MPT	9.991	10.39299	1.35951	0.77192	0.05	0	-17.83748	37.81948
PEtOx140kDa:MPT Aq200:MPT	2.011	10.39299	0.27364	0.99739	0.05	0	-25.81748	29.83948
PEtOx140kDa:MPT PnPrOx:MPT	61.99433	9.48746	9.24096	4.35603E-7	0.05	1	36.59052	87.39814

Sig equals 1 indicates that the means difference is significant at the 0.05 level.
Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

6.6.2 Figure 9: ANOVA results

ANOVAOneWay (2/06/2018 12:16:31)

Notes

Input Data

Descriptive Statistics

	Sample Size	Mean	Standard Deviation	SE of Mean
PnProx:MPT (30:70)	10	84.72126	22.80365	7.21115
PnPrOx:MPT (50:50)	15	28.09067	22.1163	5.7104
PnPrOx:MPT (20:80)	10	88.63	15.56314	4.9215

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	29495.23124	14747.61562	34.42741	1.055E-8
Error	32	13707.79006	428.36844		
Total	34	43203.0213			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.68271	0.33617	20.69706	61.56779

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
PnPrOx:MPT (50:50) PnPrOx:MPT (30:70)	-56.63059	8.44954	9.47836	3.75997E-7	0.05	1	-77.39425	-35.86693
PnPrOx:MPT (20:80) PnPrOx:MPT (30:70)	3.90874	9.25601	0.59721	0.90667	0.05	0	-18.83671	26.65419
PnPrOx:MPT (20:80) PnPrOx:MPT (50:50)	60.53933	8.44954	10.13257	6.42136E-8	0.05	1	39.77567	81.30299

Sig equals 1 indicates that the means difference is significant at the 0.05 level.
Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

6.6.3 Figure 10: ANOVA results

ANOVAOneWay (2/06/2018 12:12:08)

Notes

Input Data

Descriptive Statistics

	Sample Size	Mean	Standard Deviation	SE of Mean
80	10	80.05011	24.01393	7.59387
50	10	84.72126	22.80365	7.21115

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	109.09845	109.09845	0.19896	0.66088
Error	18	9870.07808	548.33767		
Total	19	9979.17652			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are not significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.01093	0.28423	23.41661	82.38568

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
50 kDa vs 80 kDa PnPrOx	4.67116	10.47223	0.63081	0.66088	0.05	0	-17.33047	26.67278

Sig equals 1 indicates that the means difference is significant at the 0.05 level.
Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

6.6.4 Figure 12: ANOVA results

ANOVAOneWay (2/06/2018 11:44:30)

Notes

Input Data

Descriptive Statistics

	Sample Size	Mean	Standard Deviation	SE of Mean
PnProx:MPT (30:70)	10	84.72126	22.80365	7.21115
PSecBuOX:MPT (20:80)	10	76.06963	28.2092	8.92053
PSecBuOX:MPT (30:70)	10	33.61873	15.42533	4.87792
PnPrOx:MPT (20:80)	10	88.63	15.56314	4.9215

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	19219.19458	6406.39819	14.2688	2.76125E-6
Error	36	16163.25689	448.97936		
Total	39	35382.45147			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.54318	0.29945	21.18913	70.75991

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
PsecButOx:MPT 20:80 PnPrOx:MPT 30:70	-8.65164	9.47607	1.29117	0.79806	0.05	0	-34.17285	16.86957
PsecButOx:MPT 30:70 PnPrOx:MPT 30:70	-51.10253	9.47607	7.62657	2.59371E-5	0.05	1	-76.62374	-25.58132
PsecButOx:MPT 30:70 PsecButOx:MPT 20:80	-42.45089	9.47607	6.33539	4.07285E-4	0.05	1	-67.9721	-16.92968
PnPrOx:MPT 20:80 PnPrOx:MPT 30:70	3.90874	9.47607	0.58334	0.97597	0.05	0	-21.61247	29.42995
PnPrOx:MPT 20:80 PsecButOx:MPT 20:80	12.56037	9.47607	1.87452	0.55317	0.05	0	-12.96083	38.08158
PnPrOx:MPT 20:80 PsecButOx:MPT 30:70	55.01127	9.47607	8.20991	7.32459E-6	0.05	1	29.49006	80.53248

Sig equals 1 indicates that the means difference is significant at the 0.05 level.
Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

Chapter 7

CHAPTER 7

7 OVERALL CONCLUSIONS AND OUTLOOK

7.1 ENGLISH SUMMARY PHD THESIS

In this PhD thesis, two main parts were addressed along the path towards the use of poly(2-oxazoline)s (PAOx) as matrix excipient in oral drug formulations, being a polymer synthesis part and a drug formulation part. In the first the three chapters (chapter 2-4), the polymer syntheses are described and solutions to their main challenges provided. After this, two final chapters (chapter 5-6) on drug formulation with high molar mass PAOx polymers are included and described.

For the polymer synthesis, **chapter 2-4**, it was the goal to try and find the appropriate conditions for the cationic ring-opening polymerization of 2-methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx) and 2-*n*-propyl-2-oxazoline (*n*PrOx), in order to obtain the reproducible synthesis of their corresponding high molar mass PMeOx, PEtOx and P*n*PrOx polymers on large 200 g scale. The development of a reproducible process, on large scale, for the synthesis of PAOx polymers for biomedical applications needs careful attention when PAOx as basis for future approval of PAOx by the regulatory agencies, *i.e.* European Medicines Agency (EMA, EU) and Food and Drug Administration (FDA, US).

First, in **chapter 2**, the journey towards the preparation of defined PMeOx has been described. The CROP of MeOx is found to be challenging due to the large solubility difference between the MeOx monomer and PMeOx polymer. PMeOx is even to be found insoluble in its own monomer. Attempts to find a suitable solvent to keep PMeOx in solution during its polymerization identified (*via* solvent screening) both *N,N*-dimethyl acetamide (DMA) and sulfolane as potential polymerization solvents. Because of the remarkable acceleration up to three-fold (compared to the polymerization in acetonitrile) for the CROP of MeOx in sulfolane at 140 °C, we investigated the kinetics of this polymerization reaction in more detail. A kinetic study at five different temperatures (ranging from 60 °C to 140 °C) revealed interesting accelerations from 1.7 fold at 60 °C to 3 fold at 140 °C for the CROP of MeOx in sulfolane compared to the CROP in acetonitrile. In addition, the polymerization of EtOx and 2-phenyl-2-oxazoline (PhOx) in

sulfolane were successfully performed and investigated to question the use of sulfolane as common polymerization solvent for the synthesis of PAOx polymers. Thirdly, the ability of block copolymer synthesis by sequential monomer addition in sulfolane is demonstrated, as a proof of the livingness of the CROP of 2-oxazolines in sulfolane, as well as to show the compatibility of both alkyl and aryl containing monomers with sulfolane as polymerization solvent. Finally, preliminary data proved the ability to synthesize a higher molar mass PMeOx polymer in sulfolane. Nonetheless, this last synthesis procedure needs further optimization as it was found to be difficult to handle the high boiling point of sulfolane, with regard to the acquired reaction conditions for preparing defined high molar mass PAOx polymers. In addition, the quest towards finding another extremely polar solvent, such as *e.g.* hexamethylphosphoramide (HMPT), for the CROP of MeOx can continue.

In the next chapter, (**chapter 3**) the solvent optimization for the CROP of EtOx was addressed. For this, the synthesis of PEtOx polymers has been chosen because it is the most widely studied monomer in literature (with the intention to obtain kinetic insights) for the CROP of 2-oxazolines. Also, it is remarkable that a broad solvent screening was never performed for the kinetics of the CROP of EtOx, most likely because the state-of-the-art polymerization conditions, in solvents like acetonitrile and chlorobenzene, serves the need of people working with PAOx polymers. We challenged whether the existing polymerization conditions are indeed the best there are, or whether there are improvements and other options available, regarding polymerization solvents. From the whole series of solvents (anisole, chlorobenzene, ethyl acetate, acetonitrile, 1,3-dimethyltetrahydropyrimidin-2(1H)-one (DMPU), nitrobenzene and sulfolane) that were selected for studying the kinetics of the polymerization reaction, a selection of two solvents (apart from acetonitrile and chlorobenzene) deserve special attention, being sulfolane (explanation *vide supra* or **chapter 2**) and ethyl acetate. The polymerization in ethyl acetate is selected because of its relevance towards using a green solvent for the synthesis of PAOx. Apart from the results for the CROP of EtOx in sulfolane and ethyl acetate, it was hard to generalize other trends which were observed with the other solvents, although the boiling points of the solvents appeared to correlate with the observed trends for the k_p´s.

Next, **chapter 4** focused on the large scale synthesis of defined high molar mass PAOx for biomedical applications, and more specifically for oral drug formulation (*vide infra*). While the previous two chapters dealt with mostly the optimization of the synthesis of low molar mass (< 20,000 g/mol) PAOx polymers, this chapter discussed the upscaling of the synthesis method, *i.e.* sacrificial initiator method (SIM), for the preparation of defined high molar mass PAOx polymers. This SIM, as invented by Monnery and Hoogenboom (*WO2016008817 A1,* ***2016***), was originally designed to synthesize high molar mass PAOx polymers, in a defined manner, at 1 g scale.

Nevertheless, to be able to perform the synthesis on 200 g scale, challenges such as upscaling of initiator synthesis, non-commercial monomer synthesis, purification methods and the SIM need to be addressed. The polymers that were synthesized on large scale include 25,000 g/mol, 50,000 g/mol and 100,000 g/mol PEtOx polymers and 50,000 g/mol P*n*PrOx polymers. Every synthesis of the aforementioned polymers was performed in triplicate at 60 °C on 200 g scale revealing good reproducibility. General observations include 10 % higher Đ values, with respect to the 1 g scale synthesis, but with reproducible errors and target molar masses. Also, the synthesis of a series of PEtOx (high) molar mass standards, within a molar mass range of 10,000 g/mol to 200,000 g/mol, was achieved on larger scale (50 g) at lower temperatures (42 °C). However, if they were compared to the PEtOx standards synthesized on 1 g scale, they failed to provide the same results as molar mass calibration. The latter arose the question of inefficient stirring on large scale polymerizations compared to small scale. In a final experiment, we therefore sampled on three different places in a large scale polymerization mixture, in order to verify the occurrence of a broader Đ in the PEtOx standards as a result of inefficient stirring of large scale reactions. As a conclusion, further upscaling could involve the use of *e.g.* larger reactors, mechanical stirring, *etc.* to ensure homogenous mixing and lower Đ values. Nonetheless, we were able to synthesize reproducible large scale batches of a series of PAOx polymers.

In the second part of this thesis, the polymers synthesized in chapter 2-4 are utilized for the preparation of oral drug formulations. From chapter 1 it is clear that the use of polymers in drug formulations belongs to an emerging technology in drug delivery. Here, we proposed and challenged the use of PAOx polymers as excipient for the delivery of active pharmaceutical ingredients (APIs). The influence of PAOx polymers on the drug delivery manner, *i.e.* immediate *versus* extended release, can be divided into two types of formulations, being solid dispersions (SD, **chapter 5**) and sustained release formulations (SRF, **chapter 6**).

First, in **chapter 5**, the influence of PAOx on the release and improved bioavailability of poorly water-soluble APIs has been addressed. This research originated from preliminary results by Claeys *et al.* (Macromol. Rapid Commun., 33, 1701-1707, **2012**) on the use of Aquazol® (ill-defined PEtOx) for the solubility improvement of fenofibrate (FBT). In this project, first the use of defined PEtOx for FBT SD formulations was compared to the use of Aquazol®. The tablets, prepared *via* hot-melt extrusion coupled to injection molding (HME/IM), were characterized by differential scanning calorimetry (DSC) and x-ray diffraction (XRD) and showed the absence of crystallinity of FBT. The dissolution tests performed on the FBT:PEtOx (20:80 and 40:60) SD showed similar release profiles as found for FBT:Aq200 SD (with similar drug loadings). It has to be noted that the FBT:PEtOx (40:60) SD showed the appearance of some crystallinity right after processing, which could be attributed to supersaturation of the PEtOx matrix. Furthermore, both PMeOx and P*n*PrOx

polymers with a molar mass of 50,000 g/mol, were tested for formulation with FBT. Unfortunately, the formulations containing these polymers showed high crystallinity of FBT. In correlation to the increased crystallinity found for the FBT:PMeOx (20:80) and FBT:P*n*PrOx (20:80) formulations, slower release was found compared to the FBT:PEtOx SD. Lastly, different attempts to formulate flubendazole (FLU) in a SD with PEtOx polymers were taken. Apart from solvent casting and freeze drying techniques, which were not able to formulate a SD with FLU and PEtOx, the electrospinning technique was explored, in order to get faster solvent evaporation which should lead to trapping the amorphous API in the polymer matrix. In a preliminary test, we were able to form a FLU:PEtOx (10:90) SD formulation, electrospun from a formic acid/acetic acid mixture. Characterization of the FLU:PETOx SD revealed the absence of crystallinity up to 3 months after processing. Furthermore, in the dissolution tests it is shown that 80 % FLU is released from the PEtOx matrix within the first two hours. This promising result indicates that PEtOx can serve as an immediate release matrix for SD with FLU, which should be further investigated in terms of drug loading as a function of electrospinning process.

Secondly, in **chapter 6**, the influence of PEtOx in combination with a series of more hydrophobic PAOx polymers is investigated for sustained release of extremely water soluble APIs. Throughout this study, metoprolol tartrate (MPT) is addressed as a good water-soluble model API with a relatively low melting point (± 130 °C) for the formulation in sustained release formulations (SRF). This research is also based on a preliminary study with Aquazol and 50 % of MPT loading, which showed moderately sustained release of MPT over 90 min. Again, the tablets for SRF were prepared *via* HME/IM. Within the research of this thesis, the possibility to further extend the release of MPT over a period of 24 hours, when combined with a PAOx polymer matrix, is questioned. Furthermore, the increase of the drug loading up to 80 % of MPT and the ability to slow down the release of MPT with a defined 140,000 g/mol PEtOx polymer were explored. It was shown that the MPT:PEtOx (50:50) SRF did not have any influence on slowing down the release and showed an even faster release (100 % release MPT in 1 hour), compared to the previously tested Aquazol 500 matrix. In an attempt to use the more hydrophobic P*n*PrOx matrix (with a molar mass of 50,000 g/mol) for the 50 % MPT SRF, we discovered incomplete release of only 60 % of MPT over 24 hours. Following this interesting observation, the drug loading was increased and MPT:P*n*PrOx (70:30 and 80:20) SRF were prepared and tested in dissolution tests. The release of MPT from the P*n*PrOx was found to range from being incomplete within 24 hours down to complete release within 4 hours for drug loads ranging from 50 to 80 % of MPT, respectively. Unfortunately when the MPT:P*n*PrOx SRF were tested against shear-stress stability, they showed much faster disintegration compared to the release time they provide. In other words, the tablets would not survive the *in vivo* shear-stress conditions and consequently induce faster release compared to what is shown in the *in vitro* dissolution tests, also when a higher MW P*n*PrOx of 80,000 g/mol used.

Therefore, in a final part, poly(2-*sec*-butyl-2-oxazoline) (PsecButOx, 50,000 g/mol) polymer was proposed as an alternative for the P*n*PrOx matrix to formulate MPT. The P*sec*ButOx is slightly more hydrophobic and possesses a higher T_g, resulting in higher tablet stability compared to the use of the P*n*PrOx matrix. In conclusion, it was found that the release pattern for MPT changed to 60 % release after 24 hours for a drug load of 70 % MPT. For a MPT:P*sec*ButOx (80:20) formulations complete release was observed after 9 hours. No disintegration of the corresponding tablets was observed within the provided sustained release period, for any of the P*sec*ButOx SRFs. These preliminary results have great potential and will be extended to *in vivo* studies in the near future. As a conclusion, this chapter provides a case study for the use of thermo-responsive and hydrophobic PAOx polymers as matrix excipient for the formulation of SRF with an extremely water-soluble model API, *i.e.* MPT.

Overall, this PhD thesis combined the successful optimization of the synthesis of a series of (high molar mass) poly(2-alkyl-2-oxazoline)s and the investigation on their ability to formulate (poorly) water soluble APIs in SD and SRF formulations. The synthesized PAOx polymers are varying in hydrophilicity from PMeOx to P*sec*ButOx (hydrophilic to hydrophobic) polymers, with a molar mass varying between 10,000 g/mol and 200,000 g/mol. It is important to note that the first steps were made in the direction of upscaling towards ± 1 kg scale, with the focus on improving reproducibility of the PAOx production process. In the end, this will be extremely important for the approval (by regulatory agencies) of PAOx polymers in biomedical applications, in combination with all the other tests needed, including toxicity studies, clinical trials, *etc*. Furthermore, with regard to the drug formulation part of the thesis, it is clear that PAOx polymers show potential in both immediate release (from SD) and sustained release (from SRF) formulations of a series of model APIs. This is proven on the grounds of *in vitro* dissolution tests and characterization data, such as DSC and XRD. On the other hand, the advantage of PAOx polymers in terms of tunability (structure, T_g, molar mass) has come forward in the results for SRF. Yet, up till now only model APIs were tested and formulated with (a few) PAOx polymers. Therefore, in the near future it will be crucial to perform *in vivo* dissolution tests with a selection of the best performing PAOx:API formulations based on the *in vitro* dissolution testing.

Future perspectives

As for future perspectives, tremendous efforts are shown in this thesis for the improvement and scale up of the CROP of 2-oxazolines and the application of PAOx polymers in SD and SRF oral dosage formulations. Still, there are some important challenges remaining for the near future. These challenges include for example a reduction in the overall reaction times for the preparation of high molar mass POAx, since 18 days' polymerization time for a PEtOx 140 kDa is

rather long, but not impossible, for industrial synthesis. A further scale increase from 200 g up to 2 kg scale towards 20 kg scale, is desirable and should be investigated further for which further challenges have to be addressed to maintain efficient heat and mass transfer. Especially the latter is expected to become more and more difficult at larger scale as the viscosity of the polymerization mixtures rises significantly during the polymerization, which should be addressed by moving to mechanical stirrers. Next, in this thesis mostly PEtOx and PnPrOx were produced on a 200 g scale, but there is still a range of other PAOx (co)polymers available which remained uncovered within the scope and time frame of this PhD thesis. In terms of reaction insights and the influence of different polymerization solvents for the CROP of EtOx, some trends were discovered, but further examination of possible trends has to be performed. Here, a good selection of additional solvents need to be investigated to cover the preliminary conclusions. To dissect the identified correlation with boiling point, it is recommended to directly compare a series of solvents with different polarities, but rather similar boiling points.

The pharmaceutical part of this work also offers further perspectives for the future. The first examples reported in this thesis show the applicability of different well-defined PAOx for the formulation of class I and class II APIs, with different water-solubility, in SRF and SD formulations, respectively. Nevertheless, in the future even more examples should be investigated, broadening the range of (model) APIs, formulation techniques (such as spray drying) and assessing also PAOx copolymers as excipient, in order to be able to make some valid structure-property relationships between the APIs and the PAOx (co)polymers used. Furthermore, the use of PAOx blends to tune the release rate of the API appears to be a highly promising research direction (preliminary experiments already performed for SRF). Desptie this high potential of PAOx for oral drug formations, the long-term challenge will remain to get approval for the PAOx (co)polymers for application in (oral) drug formulations. As this requires major investments to pass the regulatory trajectory, one should focus on developing formulations with PAOx that reveal unique properties that cannot be achieved with any of the approved excipients to date, such as high drug loading in SD, enhanced solubility of an API that could not yet be formulated or ultra-high drug loading SRF. If successful, this will provide thorough arguments to invest in their further use as matrix excipient for SRF and SD; and broader within the pharmaceutical and biomedical industries once one PAOx drug formulation has been approved for the clinic.

7.2 NEDERLANDSE SAMENVATTING (DUTCH SUMMARY PHD THESIS)

Dit doctoraatsproefschrift beschrijft de onderzoeksstappen en experimenten richting het gebruik van poly(2-oxazoline)s (PAOx) als hulpstof voor medicijn formulaties. Het kan verder onderverdeeld worden in twee grote delen, meer bepaald het gedeelte waarin de polymeer synthese wordt beschreven en het gedeelte rond medicijn formulaties. In beide delen van het werk zijn verscheidende uitdagingen aan bod gekomen en werd een mogelijke oplossing voor de verschillende problemen gevonden. Verder zullen in het eerste luik van deze samenvatting de drie hoofdstukken (**Hoofdstuk 2 t.e.m. 4**) rond de PAOx polymeersynthese beschreven worden. Daarna wordt een kort overzicht gegeven van de resultaten met oog op medicijn formulaties en daarbij het gebruik van hoog molecuulgewicht PAOx polymeren, welke beschreven worden in **hoofdstuk 5 en 6** van het doctoraat.

In het gedeelte van de polymeersynthese (**Hoofdstuk 2-4**), was het hoofdzakelijk de bedoeling om geschikte condities te vinden voor de kationische ring-opening polymerisatie (KROP) van 2-methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx) en 2-*n*-propyl-2-oxazoline (*n*PrOx), als basis voor de synthese van de corresponderende gedefinieerde hoog molecuulgewicht polymeren, PMeOx, PEtOx en P*n*PrOx. Hierin was het belangrijk om ook de eerste stappen te nemen richting opschaling van dit polymerisatieproces, vooral met de nadruk op reproduceerbaarheid van de resultaten. Dit is ook van uiterst belang met betrekking tot het goedkeuren (door de regulerende instanties, *Food and Drug Administration*, FDA en *European Medicine Agency*, EMA) van het gebruik van PAOx polymeren voor biomedische toepassingen, naar de toekomst toe.

Allereerst, in **hoofdstuk 2**, wordt beschreven hoe gedefinieerde PMeOx polymeren gesynthetiseerd kunnen worden. De uitdaging voor de KROP van MeOx, en het bekomen van gedefinieerde PMeOx polymeren, bestaat erin zowel het monomeer als het polymeer in oplossing te houden gedurende de polymerisatie. Dit is niet vanzelfsprekend, aangezien PMeOx niet oplosbaar is in zijn eigen monomeeroplossing wat een bulkpolymerisatie uitsluit. Via de screening van verschillende solventen werden polaire solventen zoals *N,N*-dimethylacetamide (DMA) en sulfolaan geïdentificeerd als mogelijke oplossing. Tijdens deze screening kwam sulfolaan naar voren als uitverkoren solvent voor de KROP van MeOx dankzij het versnellend effect (drievoudige versnelling t.o.v. polymerisatie in acetonitril) van sulfolaan op de propagatiestap (snelheidsbepalende stap van de KROP) voor de KROP van MeOx. Dit opmerkelijke resultaat heeft ons aangezet om kinetische studies uit te voeren bij vijf verschillende temperaturen (tussen 60 °C en 140 °C) voor de KROP van MeOx in sulfolaan. Zelfs bij 60 °C versneld sulfolaan de polymerisatie tot 1,7 keer t.o.v. de polymerisatie van MeOx in acetonitril.

Verder in dit hoofdstuk werden ook de KROP van EtOx en 2-phenyl-2-oxazoline (PhOx) uitgevoerd in sulfolaan, samen met hun kinetische studies bij dezelfde temperaturen die geselecteerd werden voor de KROP van MeOx. In het algemeen vonden we voor alle drie de monomeren een versnelde polymerisatiekinetiek. We kunnen dus stellen dat sulfolaan als universeel polymerisatie solvent voor de KROP van 2-oxazolines kan worden aangewend. In een volgende stap werd ook de synthese van een PMeOx-PhOx blok copolymeer succesvol uitgevoerd. Dit was belangrijk om te kunnen concluderen dat de CROP in sulfolaan als solvent, het levend karakter van de KROP behoud. In een laatste gedeelte werd er ook een eerste poging ondernomen voor de synthese van een hoog molecuulgewicht polymeer, meer bepaald een 50 000 g/mol PMeOx polymeer, in sulfolaan. Dit laatste is belangrijk omdat dit het eerste solvent is waarin de synthese van PMeOx met een hoog molecuulgewicht succesvol wordt beschreven. Uiteraard is hier in de toekomst nog verdere optimalisatie nodig. Verder is het belangrijk om de zoektocht naar alternatieve polaire, bv. hexamethylfosfortriamide (HMPT), solventen, voor de KROP van MeOx, voort te zetten. Dit omdat het hoge kookpunt van sulfolaan nadelig is voor de huidige hoog molecuulgewicht synthese, via statische destillatie, voor PAOx polymeren.

Als tweede luik in het polymeer synthese gedeelte, wordt in **hoofdstuk 3** de solvent optimalisatie voor de KROP van EtOx beschreven. De keuze voor het EtOx monomeer vloeit voort uit het feit dat dit het meest besproken monomeer is met betrekking tot het onderzoeken van reactie kinetiek. Verder is er nog nooit een brede screening van solventen voor deze reactie kinetisch gebeurd, waarschijnlijk omdat de goed werkende polymerisatie in acetonitril en chlorobenzeen de nood aan andere polymerisatie solventen doet verminderen. Wij stelden echter de vraag of deze condities het beste resultaat geven m.b.t. de karakteristieken van PEtOx polymeren. Twee solventen, uit een serie van apolaire tot extreem polaire solventen (anisol, chlorobenzeen, ethyl acetaat, acetonitril, DMPU, nitrobenzeen en sulfolaan), springen er bovenuit, namelijk sulfolaan (al besproken hierboven) en ethyl acetaat. De polymerisatie in ethyl acetaat is uitermate interessant voor het ontwikkelen van een ´groene´ synthese methode voor PEtOx polymeren. Uit de resultaten van de andere solventen bleek het moeilijk om een algemene trend te vinden voor de kinetiek van de polymerisatie van EtOx, hoewel het lijkt alsof er een correlatie is met de kookpunten van de solventen en de geobserveerde trends voor de propagatieconstanten.

In het laatste hoofdstuk rond polymeersynthese (**Hoofdstuk 4**), wordt de opschaling van gedefinieerde hoog molecuulgewicht PAOx polymeren beschreven. Dit is in contrast met de vorige hoofdstukken die meer de optimalisatie van PAOx polymeren beschreven met een molecuul gewicht lager dan 20 000 g/mol. Daarom staat in dit hoofdstuk de sacrificiële initiator

methode (SIM) centraal. Deze SIM werd uitgevonden door Monnery en Hoogenboom, en beschrijft de hoog molecuulgewicht synthese van PAOx polymeren, d.m.v. een zuiveringsstap over levend polymeer voorafgaande aan de polymerisatie. Deze reacties werden initieel uitgevoerd op kleine schaal (1 g). Om het verder mogelijk te maken de reacties uit te voeren op grotere schaal (200 g tot 1 kg schaal) was het nodig om rekening te houden met verschillende uitdagingen. Deze omvatten onder meer het opschalen van de initiator synthese, de synthese van niet-commerciële monomeren, de zuiveringsmethodes van monomeer en solvent, en het opschalen van de SIM. Uiteindelijk slaagden we er in om een reeks PAOx polymeren, met name 25 000 g/mol, 50 000 g/mol en 100 000 g/mol PEtOx polymeren en 50 000 g/mol P*n*PrOx polymeren, te synthetiseren op grote schaal. Tijdens deze synthese werd geopteerd om te kijken naar de reproduceerbaarheid en werd daarbij elke reactie in drievoud uitgevoerd. In het algemeen kwamen we tot de conclusie dat de dispersiteiten 10 % hoger lagen t.o.v. de synthese van de PAOx polymeren op kleine schaal. Hoewel deze ietwat polymeren minder gedefinieerd bleken te zijn, waren de standaard afwijkingen op het theoretische molecuulgewicht miniem. Bovendien zijn deze polymeren uiterst geschikt voor medicijn formulaties, aangezien ze kunnen gemaakt worden op reproduceerbare wijze. Een hogere dispersiteit is hier van ondergeschikt belang aan de reproduceerbaarheid van het proces. In een laatste aspect werd er gekeken of er ook PEtOx polymeerstandaarden met extreem lage dispersiteit (< 1,05) konden worden gesynthetiseerd op 50 g schaal. Deze PEtOx standaarden werden gemaakt met een variatie in molecuulgewicht tussen de 10 000 g/mol en 200 000 g/mol. Echter, wanneer de vergelijking werd gemaakt met de standaarden gemaakt op 1 g schaal, werd er geconstateerd dat de standaarden op 50 g schaal niet voldeden aan de strikte eisen om te dienen als massa standaarden. In de zoektocht naar een mogelijke verklaring hiervoor, werd er gedacht aan de mogelijkheid van inefficiënte homogenisatie van het reactiemengsel tijdens de polymerisatie. Om dit idee te bekrachtigen werd er een experiment opgesteld waarin op drie verschillende plaatsen in het mengsel (op grote schaal) een staal werd genomen. Hierbij werd bevonden dat vanaf 50 % conversie er wel degelijk een inefficiënte homogenisatie van het mengsel optreedt. Daarbij kan dit dus dienen als een mogelijke verklaring voor het falen van het maken van nauwer gedefinieerde polymerisatie standaarden. Een mogelijke oplossing voor verdere opschaling zou bv. kunnen zijn door te werken met grotere reactoren, mechanische roerders, *etc*.

In het tweede gedeelte van de thesis werden de gesynthetiseerde polymeren uit **Hoofdstuk 2 t.e.m. 4** gebruikt voor de bereiding voor orale medicijn formulaties. Uit de introductie (**Hoofdstuk 1**) is het duidelijk dat het gebruik van polymeren voor deze formulaties aan potentieel aan het winnen is. In dit deel, bestuderen we het gebruik van PAOx polymeren voor de bereiding van formulaties met actieve farmaceutische ingrediënten (APIs). Afhankelijk van de invloed van

de PAOx polymeren op de afgifte van het medicijn, meer bepaald onmiddellijke *versus* vertraagde afgifte, spreken we over respectievelijk vaste dispersies (SD, **Hoofdstuk 5**) of gecontroleerde afgifte systemen (SRF, **Hoofdstuk 6**).

Eerst en vooral werd in **Hoofdstuk 5** de invloed van PAOx polymeren op de afgifte en biologische beschikbaarheid van slecht wateroplosbare APIs onderzocht. Voorgaand onderzoek, uitgevoerd door Claes *et al*. (Macromol. Rapid Commun., 33, 1701-1707, **2012**), wees al uit dat Aquazol (ongedefinieerde PEtOx polymeren) de oplosbaarheidssnelheid verbeterde van fenofibraat (FBT) als model medicijn voor slecht wateroplosbare APIs. In dit project werd er daarom eerst voortgegaan op het gebruik van gedefinieerde PEtOx polymeren, met een molecuulgewicht van 50 000 g/mol, voor de formulatie van FBT in SD ter vergelijking van de bekomen resultaten voor Aquazol. De FBT:PEtOx tabletten met 20 en 40 % FBT werden, zoals in het artikel van Claeys *et al.*, gemaakt *via* smelt extrusie gekoppeld aan spuitgieten (HME/IM). Na het bereidingsproces werden de tabletten gekarakteriseerd op afwezigheid van FBT kristallen, *via* X-straal diffractie (XRD) en dynamische differentiecalorimetrie (DSC). Dit is belangrijk omdat amorfisatie van FBT een eerste aanwijzing is voor het sneller afgeven van het medicijn in een SD formulatie. Vervolgens wezen oplosbaarheidstesten uit dat de FBT:PEtOx SD met 20 en 40 % FBT inderdaad een snellere afgifte induceren van FBT, t.o.v. ongeformuleerd FBT. Als we verder de vergelijking maken met de eerder bestudeerde FBT:Aquazol SD (20:80 en 40:60), werd een gelijkaardig afgifte profiel voor FBT geobserveerd (80 % afgifte van FBT binnen het uur). Het is belangrijk om te vermelden dat de formulaties met 40 % FBT een kleine fractie API in kristallijne vorm bevatten, dat ontstaat net na de bereiding *via* HME/IM. Dit kan wijzen op een mogelijke verzadiging van de PEtOx matrix bij een medicijn belading boven de 20 % FBT. Vervolgens werden ook PMeOx en P*n*PrOx (beiden met een molecuulgewicht van 50 000 g/mol) polymeren getest m.b.t. formulatie van FBT in SD. Dit leverde echter niet de gewenste resultaten waarbij zelfs met een medicijn belading van 20 % FBT kristallisatie optrad. Er werden bijgevolg voor deze 2 andere polymeren ook geen (betere) afgifte profielen verkregen, m.b.t. de oplosbaarheidstesten. Voortgaande op de bekomen resultaten voor de PEtOx polymeren met FBT, besloten we ook flubendazol (FLU) te gaan proberen formuleren in een SD. Aangezien FLU een zeer hoge smelttemperatuur heeft (T_{smelt} > 240 °C), werd hier geopteerd om een solvent methode te kiezen voor het maken van FLU SD. In de eerste pogingen werden zowel solvent casten als vriesdrogen aangewend als technieken voor het bekomen van de formulaties met FLU:PEtOx. *Via* deze technieken was het echter niet mogelijk om een goede amorfe dispersie van FLU te bekomen. Mogelijks had dit te maken met het inefficiënt verwijderen van het oplosmiddel na formulatie. Daarom werd er in laatste instantie gekozen voor het elektrospin proces, waarbij dankzij de verhoogde oppervlak-volume verhouding zou moeten leiden tot een snellere verdamping van het oplosmiddel. Uiteindelijk werd er *via* dit proces een eerste

succesvolle amorfe formulatie met 10 % FLU en PEtOx bekomen wat bevestigd werd *via* karakterisatie met DSC. De dissolutie test (80 % afgifte van FLU binnen de 2 uur) wees uit op het bekomen van efficiënte FLU:PEtOx (10:90) SD formulaties. In de toekomst zal moeten uitwijzen of deze formulatie met FLU en PEtOx als hulpstof kan leiden tot een efficiënte formulatie met hogere medicijn belading.

Een tweede en laatste farmaceutische toepassing werd beschreven in **Hoofdstuk 6**. Hierbij werd de invloed van een serie van PAOx polymeren, met verschillende hydrofiliciteit, onderzocht als gecontroleerde afgifte matrix voor goed wateroplosbare medicijnen. In deze studie werd metoprolol tartraat (MPT) aangewend als model voor een goed wateroplosbaar medicijn met relatief laag smeltpunt (± 130 °C). Ook hier werd onderzoek naar verricht, door Claeys *et al.*, met het gebruik van Aquazol voor de formulatie van SRFs met 50 % belading. Deze MPT:Aquazol SRF, bereid *via* HME/IM, gaven volledige afgifte binnen 90 min. We vroegen ons hier af of we een (andere) hoog molecuulgewicht PAOx matrix konden gebruiken, om de afgifte te verlengen over een periode van 24 h. Hierbij werd ook de vraag gesteld naar de maximale mogelijke medicijn belading in deze SRF (tot maximaal 80 % MPT). In eerste instantie werd 50 % MPT geformuleerd met een 140 000 g/mol PEtOx polymeer. Deze MPT:PEtOx (50:50) SRF gaf echter een snellere afgifte (binnen het uur) t.o.v. de Aquazol polymeren als matrix. Vervolgens werd een 50 000 g/mol P*n*PrOx (meer hydrofoob t.o.v. PEtOx) polymeer gekozen voor het formuleren van dezelfde hoeveelheid MPT. In de bekomen resultaten voor de MPT:P*n*PrOx (50:50) SRF werd een uitzonderlijk lange termijn van afgifte ontdekt, met onvolledige afgifte van MPT (60 %) binnen 24 h tot gevolg. Gebaseerd op dit resultaat, gingen we verder kijken naar een MPT belading tot 80 %. Voor deze laatste formulatie werd volledige afgifte van MPT gerealiseerd na 4 h. Desondanks deze goede resultaten, bleken de P*n*PrOx polymeren uiteindelijk niet bestand tegen uitwendige stress (testen uitgevoerd ter simulatie van de peristaltische bewegingen in de maag). Om dit probleem te omzeilen, is de keuze gemaakt om een nog hydrofober polymeer aan te wenden voor gebruik van MPT in SRF, zijnde poly(2-*sec*-butyl-2-oxazoline) (P*sec*ButOx, met een molecuulgewicht van 50,000 g/mol) polymeren. Dankzij de iets hogere glastransitie temperatuur van P*sec*ButOx (± 55 °C t.o.v. 30 °C voor P*n*PrOx) werd er verwacht dat deze ook een gecontroleerde afgifte van MPT zouden kunnen realiseren, met een verhoogde resistentie voor uitwendige stress. De bekomen MPT:P*sec*ButOx (70:30 en 80:20) SRF, toonden zeer gunstig en gecontroleerde afgifte profielen van respectievelijk 60 % MPT afgifte na 24 h en volledige afgifte na 9 h. Verder werd er geen degradatie waargenomen tijdens de uitvoering van de stabiliteitstesten van de SRFs. Deze laatst bekomen resultaten voor P*sec*ButOx polymeren en SRF met MPT vertonen een enorm potentieel om *in vivo* testen uit te voeren in de nabije toekomst.

Samengevat kunnen we stellen dat deze doctoraatsthesis de combinatie vertoont van polymeerchemie met farmaceutische toepassingen, en meer specifiek het maken van medicijn formulaties voor orale medicijn afgifte. Voor het polymeer gedeelte werd een serie van PAOx polymeren, met verschillende hydrofiliciteit (PMeOx – PsecButOx) en molecuulgewichten, gesynthetiseerd. Hier is het belangrijk te vermelden dat de focus werd gelegd op het verkrijgen van een reproduceerbaar proces voor de synthese van deze PAOx polymeren op 1 kg schaal. Verder betekent dit een belangrijke stap in de richting van medische goedkeuring voor PAOx polymeren. Ook werd er bewezen dat PAOx polymeren effectief kunnen ingezet worden voor het formuleren van zowel SD als SRF. Echter, is het belangrijk in te zien dat dit onderzoek enkel werd verricht m.b.t. het formuleren van een aantal model medicijnen (voor zowel slecht als goed wateroplosbare medicijnen) en dat hierbij ook alleen de oplossingsprofielen in waterige buffers (*in vitro,* pH 6.8) opgesteld werden, die de toestand in het lichaam simuleren. Het zal dus nodig zijn om in de toekomst oplossingsprofielen te bepalen in bv. model dieren, om de relevantie van dit onderzoek te benadrukken en verhogen. Hierbij zal dan een selectie van de beste - in dit onderzoek vernoemde formulaties - kunnen dienen voor verdere tests.

Toekomstige perspectieven

Belangrijke stappen werden genomen inzake het verbeteren en opschalen van de KROP van 2-oxazolines en omtrent het toepassen van PAOx polymeren met betrekking tot SD en SRF orale medicijn formulaties. Ondanks het werk in deze thesis, blijven er nog een aantal uitdagingen voor de nabije toekomst. Deze uitdagingen omvatten onder meer een vermindering in de algemene reactietijd voor de productie van hoog molecuulgewicht PAOx polymeren. Dit omdat een reactietijd van 18 dagen voor een PEtOx poylmeer met een molecuulgewicht van 140 kDa wat nog steeds tamelijk lang (daarom niet onmogelijk) is, zeker in het licht van het streven naar een industrieel opschaalbare reactie. Ook zal verdere opschaling van 200 g naar 2 kg tot zelfs 20 kg een prioriteit moeten worden voor verder onderzoek, met de nadruk op hoe efficiënte massa en warmte transfer kan gebeuren op een nog grotere schaal. Vooral de warmte transfer zal moeilijker controleerbaar zijn op grote schaal omdat de viscositeit enorm toeneemt tijdens de polymerisatie, wat zal kunnen beïnvloed worden door over te schakelen op mechanisch roeren van het reactiemengsel. Verder, is er in deze thesis enkel de nadruk gelegd op het produceren van PEtOx en P*n*PrOx polymeren op 200 g schaal. Het is natuurlijk zo dat er nog een grote variëteit aan andere (functionele) PAOx (co)polymeren kan gesynthetiseerd worden, hoewel deze niet werden gemaakt of behandeld binnen het tijdsbestek van deze thesis. In termen van verdere reactie inzichten, werd er al heel wat verwezenlijk met betrekking tot het effect van het polymerisatiesolvent op de reactie. Hoewel sommige trends werden geobserveerd, is het nog steeds moeilijk om algemene conclusies te vormen inzake de invloed

dat deze solventen hebben gecorreleerd aan hun fysicochemische eigenschappen. Hiervoor zullen een reeks extra solventen verder onderzocht moeten worden om de eerste conclusies, die gemaakt werden in deze thesis, verder te staven. Om de invloed van het kookpunt van het solvent verder onder de loep te nemen, zou het aan te raden zijn om een reeks solventen met vergelijkende kookpunten, maar verschillende polariteit, te onderzoeken voor de KROP van EtOx.

Het farmaceutische luik van deze thesis opent ook perspectieven naar de toekomst. Eerst en vooral tonen we de mogelijkheid van het gebruik van goed-gedefinieerde PAOx polymeren voor de formulatie van klasse I en II APIs, met een verschillende water-oplosbaarheid, in respectievelijk SRF en SD formulaties. Dit neemt niet weg dat er in de toekomst meer onderzoek moet gedaan worden op bv. een uitbreiding van het aantal APIs, verschillende formulatie technieken (bv. ook kijken naar sproeidrogen) en het onderzoeken naar het effect van PAOx copolymeren op de formulatie van APIs. Dit laatste, zou er dan ook voor kunnen zorgen dat we meer structuur-eigenschapsrelaties kunnen maken tussen de geformuleerde APIs en daarbij gebruikte PAOx copolymeren. Het nader onderzoeken van formulaties met PAOx polymeer blends kan dan op zich weer een andere manier zijn om afgifte van het API als dusdanig te finetunen. Hier zijn ook al preliminaire testen aan het onderzocht worden voor SRF. Desondanks het hoge potentieel van het gebruik van PAOx polymeren voor orale afgifte systemen zal de goedkeuring/regularisatie van hun gebruik als excipient een belangrijke uitdaging worden op lange termijn. Deze goedkeuring vraagt een grote investering om het regularisatieproces te doorkruisen enerzijds, daar anderzijds we moeten focussen op het onderzoeken van formulaties met PAOx polymeren die unieke kwaliteiten naar voren brengen. Deze kwaliteiten en unieke eigenschappen hebben dan vooral te maken met betrekking tot hoge medicijn-belading in SD, verbeterde oplosbaarheid van een API dat met de huidige markt excipienten niet formuleerbaar blijkt of het kunnen formuleren van zeer hoog API beladen SRF. Indien deze kwaliteiten en eigenschappen succesvol kunnen worden aangetoond, zal dit zich ook vertalen naar voldoende argumenten om te investeren in PAOx polymeren als excipient van de toekomst voor SD en SRF. Uiteindelijk kan het goedkeuren van één PAOx formulatie in de clinical trials ervoor zorgen dat er een breder gebruik komt van PAOx polymeren in farmaceutische en biomedische toepassingen, op langere termijn.

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